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GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

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CORRIGENDA

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Page 26, line 12, for "B?/B^A D; C/c" read "Bd/B^A D; C/c."

Page 30, figure 10, for "left side blue checker, *S*" read "left side blue checker, *s*."

Page 195, line 2 in "Addendum," for "(1939)" read "(1938)."

CALVIN BLACKMAN BRIDGES

CALVIN BLACKMAN BRIDGES was born in Schuyler's Falls, New York, on January 11, 1889, and died in Los Angeles December 27, 1938. His father, LEONARD VICTOR BRIDGES, who was brought up on a small farm near Plattsburg, married CHARLOTTE AMELIA BLACKMAN in 1887, and Calvin was their only child.

His mother died when Calvin was two years old. His father died a year later. The Grandmother Bridges had already brought up a large family but considered it her duty to take charge of Calvin. He went to a small district school when it was convenient. When he was about 14 years old his grandmother insisted that he should be "educated," and he was allowed to go to Plattsburg. He was not ready for high school and had to spend two preliminary years in grammar school. He graduated from high school in 1909 when he was 20 years old. He had made a fine record. During the years in Plattsburg he drove to school part of the time with neighbors and worked and lived much of the time in town. He worked as "printers devil" on the Plattsburg Press, and it was said "there is a boy who is going to amount to something." When he had spare time he was always at his books, but he was a "regular boy," good-natured and obliging.

One summer during his high school years he worked on a geological survey of Valcour Island with Professor GEORGE H. HUDSON of the Plattsburg Normal School. There is extant a letter by Professor HUDSON written when Calvin applied for a scholarship at Columbia University in which he says: "He is in many respects a very remarkable young man. He has always kept on the honor roll of his school and yet read very extensively outside of the lines of his school work." The list of the books he read, in Calvin's handwriting, exists and it certainly covers a very wide range of subjects. HUDSON also says: "He has the questioning mind, is apt in the forming of hypotheses and quick to see when they are weak. His great desire is to undertake research work."

At the end of his high school work Calvin was urged to try the regional examination for a four year scholarship at Cornell which he passed. He also took an examination for a one year scholarship at Columbia University and passed this also. Wishing to live near his aunt, Mrs. BILLINGS, who lived in New Jersey, he accepted the Columbia scholarship. During his three years at college he received some aid from scholarships, but earned much of his living in other ways. During one of the summers he tutored two young boys and during another he went "on the road" to sell a book entitled "Standard Dictionary of Facts."

My first contact with BRIDGES was in 1909 when he took a course I gave in General Biology and another in Embryology (1910); and later, as a

graduate student, he took my course in Experimental Zoology, largely devoted to genetics, and a course in Experimental Embryology. He attended also Professor E. B. WILSON's course on The Cell.

BRIDGES graduated (B.S.) from Columbia College in 1912. In the same year he married GERTRUDE F. IVES, and is survived by his wife and three children, Philip, Betsey and Nathan. From 1910 to 1915 he served as part-time assistant in my work on Drosophila. In the course of this work (MORGAN and BRIDGES 1913) certain exceptions turned up that BRIDGES began to study intensively. In 1913 he published briefly his results under the title of "Non-Disjunction of the Sex Chromosomes of Drosophila." A much more extended paper appeared in 1916 entitled "Non-Disjunction as Proof of the Chromosome Theory of Heredity." This work, offered as his doctoral dissertation at Columbia University, included not only genetic evidence but corresponding evidence from a study of the chromosomes that tallied with the genetic results. This paper went far towards convincing skeptics and conservatives that chromosomes are the bearers of genetic factors. It is true there was abundant evidence before 1916 showing that chromosome behavior furnishes an interpretation of heredity. It is today hard to believe that it was nearly ten years before this relation was generally accepted.

Sex-linked inheritance, as connecting genetic interpretation with known transmission of the sex chromosomes, had been established since 1910. The interpretation of crossing over, supported by less certain cytological evidence, had also been advanced in 1910, and covered what seemed to be exceptions to MENDEL's laws. Crossing over opened up a new field of research in inheritance, and for 30 years formed the material on which much of BRIDGES' work rested. He greatly improved the technique of locating the genes, and in the course of the following years he so thoroughly built up the genetic maps that these stand today as the most complete data we have of the location of the genes in the chromosomes.

During 1915–1916 BRIDGES held a University Fellowship at Columbia and from 1915 until his death he was associated with the Carnegie Institution of Washington first as assistant (1915–1919) and later (1919–1938) as staff member.

From 1916 to 1928 he was a member of the Carnegie group of investigation working at Columbia University, and from 1928 to 1938 at the California Institute of Technology. The annual grants from the Carnegie Institution of Washington contributed greatly towards the success of the genetic work on Drosophila.

In 1916 BRIDGES and I brought together the then known data on the mutants and linkage relations of the first or X chromosome of Drosophila, in 1919 those of the second chromosome were published, and in 1923 we

published the data which had accumulated on the third chromosome group of mutants. Later (1935) BRIDGES gave a complete account up to date of the fourth chromosome (Journal of Biology, Moscow, U.S.S.R.).

The first gynandromorph of Drosophila was discovered in 1910. From that time onward such exceptions were watched for and recorded. In time Bridges and I had a considerable number on hand which we described in a Carnegie Publication in 1919. Bridges reported (1939) that we had at that time studied "about 100 gynandromorphs and found that the maternal X was eliminated about as often as the paternal X. In experiments in which all flies were counted 40 gynandromorphs occurred in 88,000 flies." In addition to our explanation of elimination of one of the X chromosomes, during early development, a few other exceptions were found that called for a different explanation; namely, the presence of two separate nuclei and reduction products in the egg.

Later BRIDGES discussed a few individuals showing spots on the body which, from genetic evidence, were composed of haploid cells, and, in some of these mosaics, the regions included body parts that enabled one to diagnose their sex. The spots were clearly female in constitution, and this was surprising at the time since in other insects, where haploid individuals were known (bees, etc.), they were males. BRIDGES' finding in Drosophila was, however, consistent with the female sex formula: two X plus two sets of autosomes. In the spots the same balance is present, that is one X and one set of autosomes, so that the haploid somatic tissue is female.

During the years 1921–1925 BRIDGES made an extensive study of certain types of intersexes in Drosophila. He showed clearly by cytological analyses that they were due to chromosome aberrations of a type that had not been established previously for intersexes of other insects. The intersexes showed complex mixtures of male and female parts. In the first culture in which they were found (1920) there were 37 of them in addition to 9 regular males and 96 females. They were completely sterile but certain of their sisters, when bred, gave intersexes and were shown by genetic and cytological evidence to be triploid (3N) females. The breeding experiments with such females showed that a certain proportion of their mature eggs contained one full set of chromosomes, one X and one of each autosome, and, in addition, part or all of one extra set. Those mature eggs that contained a diploid set of chromosomes, would, if fertilized by a normal X-bearing sperm, again give 3N females, and if fertilized by a Y sperm would give intersexes. The formula for the intersexes is $3A+2X+Y$. They differ from standard females only in having an extra set of autosomes, and this fact, as BRIDGES pointed out, "proved that autosomes (A) are as much determiners of the normal sex differences as are the so-

called sex chromosomes. Autosomes turn the scale toward maleness." This idea of genic balance he had already developed in 1921 in connection with changes induced by loss or gain of a fourth chromosome. The theory was applied to the interpretation of certain other abnormal-appearing types of individuals, notably superfemales and supermales. A superfemale, he showed, arises when an individual has three X chromosomes and two autosomes. A supermale arises when one X and three sets of autosomes are present. Three types of females may exist in which the balance between X's and autosomes is the same as in the normal (2X+2A) female, namely, 3X+3A and 4X+4A, besides the normal. These conclusions of BRIDGES were not theoretical speculations, but in every case the interpretation rested on genetic evidence, and in triploids and diploids on a cytological demonstration of the presence in the individual in question of the constellation of the chromosomes that was postulated.

BRIDGES paid a great deal of attention to the problem of genic balance, pointing out that it is fundamental for an understanding not only of the balance concerning sex, but for all other characters as well. The fact is that today there is demonstrable evidence that there is not a single gene for femaleness and another for maleness but several, perhaps many, genes distributed through the chromosomes and affecting the development of certain characters in one way or in the opposite way. This conception in its broadest aspects has always been insisted on by the group working on the genetics of Drosophila, although it is also true that a change in a single gene often leads to striking changes in the individuals containing such a mutant gene. They argued that both the old and the new gene influence the end result, not by acting alone but by collaborating with other genes, and, in the last analysis, with all or most of the genes to different degrees. Each gene is thought of as a differential.

BRIDGES' early discovery (1917) that certain genetic data could be interpreted as due to deficiencies in the chromsome construction has led in recent years to a factual demonstration of such deficiencies. In some of his latest work (1937–1938) he made use of this discovery in the interpretation of overlapping deficiencies in analyzing the characteristics of certain mutant types. It would be hard to find in the history of genetic research a more convincing demonstration of the combination of factual evidence and masterly interpretation of it. As early as 1919 BRIDGES described "duplication" as a chromosomal aberration, and here, as in his other work, his conclusions rested not on vague hypotheses but on experimental proof. Much later he also reported the occurrence of "repeats" in the normal chromosome which will have to be seriously considered in future interpretations of certain types of genetic behavior.

The many interesting problems connected with losses (deficiencies)

and additions (duplications) of groups of genes present interesting problems in which genic balance is involved, and the possibility, that now exists, of detecting deficiencies and duplications in the salivary chromosomes and correlating the observations with the genetic location of mutant genes is well under way, and during the last two years of his life, was receiving BRIDGES' close attention.

In 1925 the data, that had been collected in the course of genetic experiments, was brought together under the title "The Genetics of Drosophila," by T. H. MORGAN, C. B. BRIDGES, A. H. STURTEVANT. Not until 1934, when the first number of Drosophila Information Service edited by BRIDGES and DEMEREC appeared, was a similar summary made. BRIDGES spent a tremendous amount of hard work in summarizing the data, particularly those of the stocks that the Carnegie group had built up at Pasadena, and the reports include also much unpublished data that BRIDGES himself had on hand. Eleven numbers of Drosophila Information Service have appeared, the latest in 1939.

In recent years BRIDGES spent much time in correlating the loci of the genetic maps with the bands of the salivary maps. He made an elaborate study of the salivary chromosomes, and more than doubled the previously known number of bands. His maps have become the standard ones for *Drosophila melanogaster*. As I have pointed out elsewhere, the identification of the salivary bands with the loci on the genetic map would not have been possible were it not that during the preceding twenty-three years the genetic (crossover) maps had been built up to a point where such comparisons have a real demonstrable basis. While many workers had contributed to bring the genetic maps to their status of 1933, it was BRIDGES in particular who had made a more detailed and critical study of the maps than had any other one of his contemporaries.

As a member of the Carnegie group each year's progress was reported in the Year Book of the Carnegie Institution of Washington. These twenty-three reports give in briefest summary the results that BRIDGES had obtained. Whether the elaborate data on which these reports rested, can ever be fully utilized is questionable; but BRIDGES accomplished so much other work, that these will not be needed to place him amongst the leading geneticists of his time.

T. H. MORGAN

CYTOLOGICAL AND DEVELOPMENTAL STUDIES OF HYBRIDS BETWEEN MEDICAGO SATIVA AND A DIPLOID FORM OF M. FALCATA

GEORGE F. LEDINGHAM
University of Wisconsin, Madison, Wisconsin[1]

Received July 21, 1939

THE two species, *Medicago sativa* and *M. falcata*, are commonly considered as varieties of one highly polymorphic species, since they intercross readily (WALDRON 1920, TYSDAL and WESTOVER 1937) and all intergradations between the two main types may be found. *M. falcata*, however, exists in both the diploid and tetraploid form (FRYER 1930), and it is only as the tetraploid that it crosses readily with the tetraploid *M. sativa*. Heretofore, no successful cross between the diploid *M. falcata* and *M. sativa* has been reported. The present paper describes two triploid hybrids formed when the diploid form of *M. falcata* was the male parent, and several tetraploid hybrids formed when it was the female parent in crosses with *M. sativa*.

Histological studies were made in an endeavor to find the origin of the tetraploid hybrids and an explanation for the frequent failure of development of the triploid hybrids. Conditions in the developing ovules following crosses between plants of different chromosome number in Medicago are similar to those in Avena (KIHARA and NISHIYAMA 1932), Triticum (WAKAKUWA 1934, BOYES and THOMPSON 1937) and Nicotiana (KOSTOFF 1930). Fertilization occurs, but apparently the relation between the nuclear and cellular divisions in the endosperm is upset, so that many wrinkled, inviable seeds are formed when the female has the lower chromosome number but fewer, plumper, more viable seeds when the female has the higher chromosome number. The causes of the failure and the reciprocal cross differences in Medicago seem to lie in the endosperm as in the plants previously studied, though Medicago seeds normally have no endosperm at maturity.

MATERIALS AND METHODS

A yellow-flowered variety of alfalfa of unknown origin was obtained through A. H. WRIGHT at the University of Wisconsin. R. A. BRINK found that it did not cross readily with *Medicago sativa*. The somatic chromosome number was shown to be 16 by D. C. COOPER. The variety is winter hardy and may originally have been introduced from Siberia. It has been identified as *M. falcata*, a determination confirmed by H. A. SENN, Assistant Dominion Botanist, Ottawa, Canada. The plants are closely similar to and cross freely with those described by FRYER (1930) as the diploid form of *M. falcata*.

[1] Paper from the Department of Genetics, Agricultural Experiment Station No. 253.

Plants of the tetraploid *Medicago sativa*, the diploid *M. falcata* and several other diploid species of Medicago were grown in the greenhouse during the winters of 1936, 1937, and 1938. Artificial illumination was used to force the plants into flower in April. A few plants, transplanted from the field in September, 1938, flowered in October. Emasculations were made by an electrically driven vacuum pump following the method described by KIRK (1930). Pollinations were made by applying pollen, collected on emery paper, mounted on a toothpick (BRINK 1934). The seeds matured in about thirty days and were then grown in a flat before the seedlings were transplanted to the field.

Root tips for somatic chromosome counts were collected directly into Karpechenko's modification of Navashin's fixing fluid. Bud material for the study of chromosome behavior during meiosis and pod material for the study of the development of the embryo and endosperm were immersed in Carnoy's solution (3:1) for about a minute before placing in the Karpechenko fixative. The material, after being allowed to fix for about 24 hours, was dehydrated, cleared in cedarwood oil, embedded and sectioned. Meiotic and root tip materials were stained by the iodine-crystal violet-picric acid method. The ovary material was stained with Heidenhain's iron alum haematoxylin. Some meiotic material collected in Carnoy's solution was studied in aceto-carmine. Pollen grains were examined in lacto-phenol-methylene blue.

DEVELOPMENT FOLLOWING RECIPROCAL CROSSES

Two triploid hybrids were obtained in 1937 by R. A. BRINK after crossing a particular strain of *Medicago sativa*, known as K40, by the diploid *M. falcata* (table 1). Although over 700 flowers of *M. sativa* were pollinated by *M. falcata* in 1938, no more triploid hybrids were obtained. Since the K40 strain was not used in 1938 it may be possible that different lines of *M. sativa* differ in their crossability (BACKHOUSE 1916, MANGELSDORF and REEVES 1931, KARPECHENKO 1937) with the diploid form of *M. falcata*. When *M. falcata* was used as the female no triploid hybrids were obtained in either 1937 or 1938, though the ovaries started to develop in practically every case. About 60 percent of the ovaries matured but they contained only small, shrunken, inviable seeds and a few viable seeds which proved to contain tetraploid hybrids. A study of the differences in the reciprocal crosses was made in order to understand more clearly the reasons for the results.

A summary of the observations of the embryos developing following the cross *M. falcata* ♀ × *M. sativa* ♂ is given in table 2. Pollen tubes are found at the base of the ovaries, and 50 percent of the ovules are developing in the material collected 32 hours after pollination. The stage of de-

velopment is usually a 2-cell proembryo accompanied by 8 or 16 free nuclei in the endosperm. Seven chromosome counts obtained at metaphase of the first nuclear division in the proembryo show $2n = 24$ (figures 1, 2, also 5, and 9), proving that cross fertilization of the diploid by the tetraploid takes place.

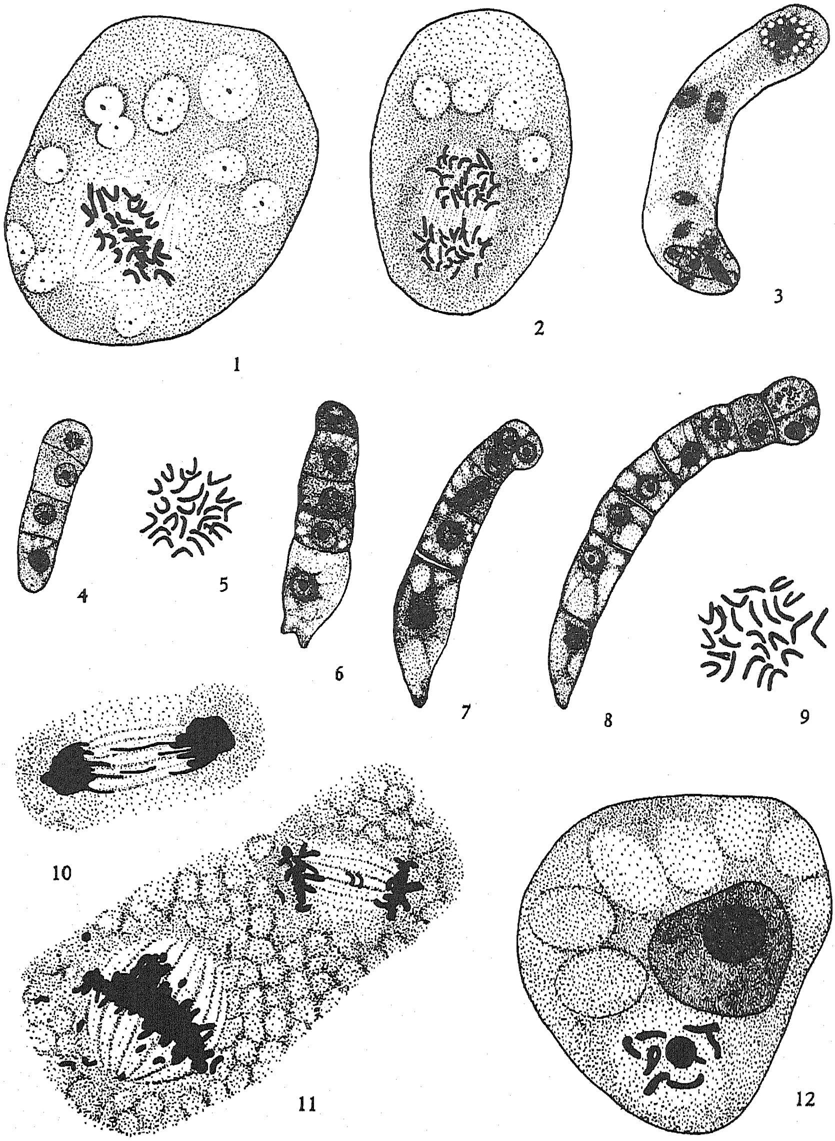
TABLE 1

Controlled greenhouse crosses of M. falcata ($2n = 16$) and M. sativa ($2n = 32$).

CROSS	YEAR	NO. FLOWERS POLLINATED	NO. PODS FORMED	PERCENT PODS FORMED	NO. SEEDS FORMED	NO. PLANTS GROWN	SOMATIC CHROMOSOME NUMBER
M. falcata ($2n = 16$) × *M. sativa* ($2n = 32$)	1937	—	—	—	11	5	16
						4	32
	1938	408	247	60.5	39	13	32
						8	—
M. sativa ($2n = 32$) × *M. falcata* ($2n = 16$)	1937	—	—	—	5	2	24
						3	32
	1938	774	30	3.9	28	12	32
						6	—

Embryos of *M. falcata* × *M. sativa* collected from two to six days after pollination show progressive stages of development (figures 3 to 8) as in *M. sativa* material (COOPER 1935). The rate of development, however, is considerably slower. In five-day material the proembryo varies from two to ten cells, having no more than four cells in the embryo proper in any case (table 2), whereas *M. sativa* material reaches the same stage of development in three days. The number of ovules developing decreases rapidly after six days but a few triploid embryos continue to develop and show traces of cotyledonary bulges at 18 days. Degeneration does not occur conspicuously at any one stage or in any particular part of the ovule, but all cells and nuclei become less deeply staining. Usually the inner integument becomes more meristematic in appearance and increases slightly in size and number of cells.

The endosperm develops rapidly at first, and 16 free nuclei are often present 32 hours after pollination, whereas only four or eight occur normally in *M. falcata*. Assuming that fertilization does not take place until 24–27 hours after pollination, as in *M. sativa* (COOPER 1935), it



FIGURES 1–12

Camera lucida drawings of embryo and endosperm development in *Medicago falcata* ♀ (n = 8) × *M. sativa* ♂ (n = 16), from paraffin serial sections. Magnification reduced one-half in reproduction to ×165 for figure 3, ×360 for figures 4, 6, 7, and 8, and ×1625 for the remaining figures.

FIGURE 1.—First nuclear division of the proembryo in metaphase, 2n = 24.

FIGURE 2.—First nuclear division of the proembryo in anaphase, 2n = 24.

FIGURE 3.—Embryo sac 2 days after pollination, two-cell proembryo, eight nuclei in division in the endosperm, chalazal nuclei more advanced.

FIGURE 4.—Four-cell proembryo at four days.

FIGURE 5.—2n = 24 chromosomes from apical cell of proembryo shown in figure 4.

TABLE 2

Embryo development following the cross Medicago falcata ♀ ($n=8$)×*M. sativa* ♂ ($n=16$).

AGE AFTER POLLINATION	TOTAL OVULES	PERCENT OVULES DEVELOPING	TOTAL NUMBER OF CELLS											EMBRYOS OF KNOWN CHROMOSOME NUMBER	
			PROEMBRYO					EMBRYO PROPER							
			1	2	3	4	6	2	4	8	16	32	MANY	24	32
32 hours	521	50.1	61	200										7	—
2 days	196	27.0	19	34										3	—
3 days	195	33.8	7	38	15	9								2	—
4 days	189	38.6		11		51	12	8						2	—
5 days	334	38.0		14		39	30	39	2					8	—
6 days	44	47.7				2	1	2	4	9	2			1	—
8 days	72	13.9							1	2	4	3		—	—
11 days	72	10.0									2	5		—	—
18 days	99	10.0		1		1	2						6	3	1

must be concluded that the early development of the endosperm is rapid and exceeds that occurring in normal *M. falcata* material. The nuclear divisions in the endosperm, however, are not entirely regular (figure 10), and in older material occur less frequently. The nuclei attain various sizes as shown in figure 11, but are usually in the resting stage with irregular shape and numerous nucleoli. The endosperm may be completely resorbed before any sign of degeneration is evident in the embryo or integumentary cells.

One embryo in the 18-day material, though smaller than normal *M. falcata* embryos of similar age, is better developed than any *falcata* × *sativa* triploid embryo observed. It has prominent cotyledonary bulges, and 32 chromosomes were counted in several cells (table 2). The endosperm surrounding this tetraploid embryo differs conspicuously from that accompanying the triploid embryos in being cellular and normal in appearance. It is probable that this embryo would have become a tetraploid hybrid.

When *Medicago sativa* ♀ ($n=16$) is crossed with *M. falcata* ($n=8$), the flowers usually drop off in from three to five days (table 3). Thirty-two hours after pollination approximately 25 percent of the ovules are developing (figure 12), and the pollen tubes extend about half way to the

FIGURE 6.—Five-cell proembryo at five days.
FIGURE 7.—Seven-nucleate proembryo at four days.
FIGURE 8.—Nine-cell proembryo at five days.
FIGURE 9.—$2n=24$ chromosomes from the cell in division in the embryo shown in figure 8.
FIGURE 10.—Irregular nuclear division in the endosperm 30 hours after pollination.
FIGURE 11.—Large and small endosperm nuclei in division at six days.
FIGURE 12.—Delayed fertilization, showing the eight chromosomes of the male gamete in advance of the female nucleus when *M. sativa* ♀ ($n=16$)×*M. falcata* ♂ ($n=8$).

base of the ovaries. In two-day material fertilization is complete, and slightly more development has taken place in some ovules. Three-day material is no further developed, and many ovules have begun to degenerate. In the four- and five-day material the embryo sac is completely collapsed, and no sign of further development can be found, except in two cases which are probably the result of selfing.

TABLE 3

Embryo development following the cross Medicago sativa ♀ $(n=16) \times$ *M. falcata* ♂ $(n=8)$.

HOURS AFTER POLLINATION	NUMBER OF FLOWERS	TOTAL NUMBER OVULES	NUMBER DEVELOPING OVULES	DEVELOPMENT	
				ZYGOTE CELLS	ENDOSPERM NUCLEI
32	23	219	53	1–2 proembryo	2–4
48	25	212	94	1–3 proembryo	4–16
72	31	307	91	1–3 proembryo	4–16
96	20	214	1	2 embryo proper	16
120	79	870	1	16 embryo proper	16

Chromosome counts were obtained in six cases. The proembryo, in the four cases in which division was observed, has $2n=24$. The endosperm in two cases has 40 chromosomes, only 8 of which are from the male parent. These counts prove that fertilization takes place. Development, however, is very slow; the nucellus does not disappear as in the normal *sativa* material but seems to increase in size and cytoplasmic density.

TRIPLOID HYBRIDS

The two triploid hybrids (figure 13) which were formed following the cross *M. sativa* × *M. falcata* (table 1) are intermediate between the parental types. The leaflets are longer and more serrate at the apex than are those of *M. sativa.* The flowers are variegated, and the color becomes more yellow as the flowers age. The two triploid hybrids differ considerably from each other. One is larger with long racemes and is more easily propagated vegetatively. The other is smaller with short racemes, darker flowers and leaflets which are more acute at the tip. The fertility and meiotic behavior of the two plants, however, is approximately the same.

The chromosome behavior in microsporogenesis is typical for autotriploids. Several scattered univalents are usually present during the first metaphase (figure 14), and sometimes eight bivalents and eight univalents can be recognized (figure 15). During the first anaphase five to eight univalents (figures 16 and 17) lie scattered in the spindle where they sometimes divide. The lagging chromosomes, not included in the two

nuclei at telophase, form micronuclei (figure 18). The second meiotic division is more regular (figure 19), and four microspores of approximately equal size are formed (figure 23). Univalents, divided during the first division, lag on the homoeotypic spindles (figure 20).

When all or nearly all of the univalents are included in one of the two nuclei during the first division, two large and two small nuclei are formed during the second division (figure 21). Micronuclei are frequently present after the second division (figure 22). One or two extra microspores, smaller than the other four, may be formed (figures 24 and 25), and 20 percent of one count of 224 microspores were of this extra type. The microspores are frequently of irregular size and shape (figure 26). Pollen grains from

TABLE 4

Medicago sativa-falcata derivatives obtained by backcrossing the triploid hybrid to the parental types.

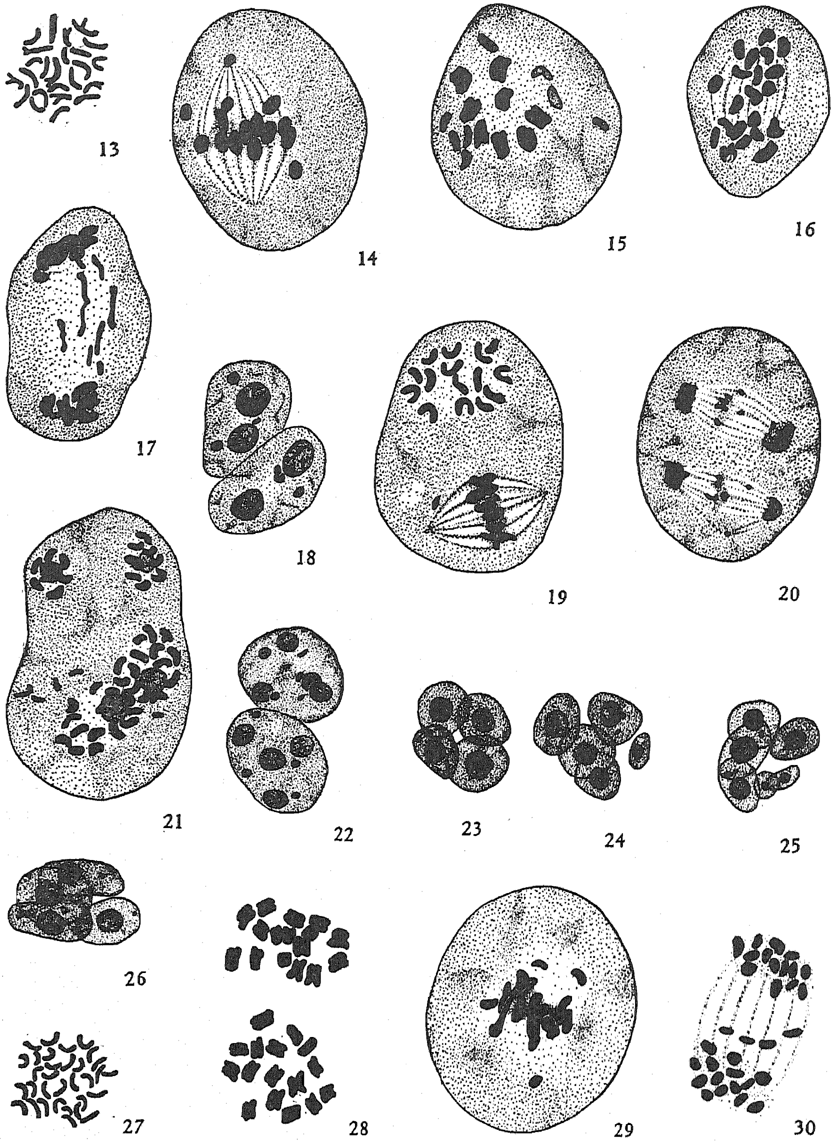
CROSS*	NUMBER INFLO-RESCENCES	TOTAL NUMBER FLOWERS	TOTAL PODS FORMED	PERCENT FLOWERS FORMING PODS	TOTAL SEEDS FORMED	SEEDS PER 100 OVULES	PLANTS OBTAINED	PERCENT GERMI-NATION
24×16	70	802	48	5.98	44	0.55	15	34
16×24	8	61	1	1.64	1	0.16	1	100
24×24	17	201	0	—	0	—	0	—
24 self	23	263	0	—	0	—	0	—
24×32	183	2187	157	7.18	80	0.36	31	39
32×24	38	308	18	5.84	26	0.84	11	46

* Parent plants are indicated by their somatic chromosome number. The female is given first.

the open flowers are greatly shrunken but about 20 percent contain deeply staining protoplasm, suggesting that the triploids would show some fertility.

The results of selfing and intercrossing the two triploid hybrids as well as outcrossing them both as male and female, to the diploid *falcata* and the tetraploid *sativa* types are summarized in table 4. No seeds were formed when the triploids were selfed or intercrossed, though many triploid flowers not included in the table were artificially self pollinated by rolling between thumb and fingers. In addition to the sterility arising from the irregular chromosome behavior, it is possible that self-incompatibility (BRINK and COOPER 1938) has increased the sterility of the triploids.

Sixteen plants were grown following hybridization between the triploid hybrid and the diploid *M. falcata*. One arose when the triploid was used as the male parent. It is of the *falcata* type but may not be the result of selfing since *M. falcata* plants are highly self-incompatible. Of the remaining plants, eight have 16, three have 17, and one has 18 chromosomes. The



FIGURES 13–30

Camera lucida drawings of triploid and tetraploid microsporogenesis, from aceto-carmine smears, and somatic chromosomes from paraffin sections. Magnification reduced one half in reproduction to ×740 for figures 18, 22, 23, 24, 25, and 26 and to ×1625 for the remaining figures.

FIGURE 13.—Triploid hybrid, root tip chromosomes, $2n=24$.

FIGURE 14.—Triploid first metaphase, lateral, several univalents off plate.

FIGURE 15.—Triploid first metaphase, polar, $8_{II}+8_{I}$.

FIGURES 16, 17.—Triploid first anaphase, lateral, lagging of chromosomes.

FIGURE 18.—Triploid interphase, several micronuclei in each pollen mother cell.

FIGURE 19.—Triploid second metaphase, polar and lateral, regular.

chromosome behavior is usually regular. Most of the plants show a rather strong influence of the *sativa* type. The flower color varies from pale yellow to variegated with a predominance of yellow. The pods are usually more sickle shaped than in the original *falcata* type.

A considerable number of seeds were obtained when the triploid was crossed with *M. sativa*. Few plants, however, were obtained when the triploid was the male parent. They are of the *sativa* type and are probably the result of selfing or contamination of the *sativa* female parent. About thirty plants were obtained from seeds formed on the triploid plants following outcrossing with *M. sativa*. While variable, these plants, in general, conform to the *sativa* type. The flowers on nearly all of them, however, show some trace of yellow pigment and the pods are more loosely coiled than in *M. sativa*. Chromosome counts from sixteen of the plants range from 30 to 36 but are usually 32, in which case the chromosome behavior is regular.

The above results show that the female gametes of the triploid hybrids which have produced viable plants on outcrossing have 8 to 22 chromosomes. Apparently only those with 8 to 10 chromosomes are functional when the male parent is *M. falcata*, whereas those with 14 to 22 chromosomes are functional when the male parent is *M. sativa*. Evidently macrospores with a wide range in chromosome number are formed but few form viable gametes and their development depends partly on the chromosome number of the male gamete. The intermediate variable character, the high fertility, and the chromosome number of the *falcata* and *sativa* outcross derivatives of the triploid hybrids suggest that the *falcata* and the two sets of *sativa* chromosomes pair freely.

TETRAPLOID HYBRIDS

The cross, *Medicago falcata* ♀ ($n=8$) $\times$ *M. sativa* ♂ ($n=16$), gave seventeen plants (table 1) which, on cytological examination, were found to have 32 as the somatic chromosome number (figure 27). Explanations in terms of haploid or diploid androgenesis do not apply since the flowers are

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FIGURE 20.—Triploid late second anaphase, with lagging chromosomes.

FIGURE 21.—Triploid late second anaphase, following an unequal distribution of univalents during the first division.

FIGURE 22.—Triploid pollen mother cells, after the second division, with micronuclei.

FIGURE 23.—Triploid, four typical microspores.

FIGURES 24, 25.—Triploid microspores, with one or two extra cells.

FIGURE 26.—Triploid microspores, four cells of irregular shape.

FIGURE 27.—Tetraploid hybrid, root tip chromosomes, $2n=32$.

FIGURE 28.—Tetraploid first anaphase, regular, $16+16$.

FIGURE 29.—Tetraploid first metaphase, lateral, several univalents.

FIGURE 30.—Tetraploid second anaphase, lateral, four lagging chromosomes.

variegated, as would be expected in true hybrids. The plants are evidently tetraploid hybrids which have received twice the normal number of chromosomes from the diploid female parent. The flower color is closer to that of the diploid yellow-flowered parent while the size of the flowers and leaves is closer to that of the tetraploid parent. The 25 plants (including eight in which the chromosome number was not determined) are the result of 17 different crosses involving six different *sativa* plants and nine different diploid *falcata* plants. No more than one viable seed was obtained from any one flower pollinated so the increased chromosome number must have occurred in individual ovules.

The tetraploid hybrids when selfed formed only 0.1 seed, while on intercrossing different plants of this type 2.1 seeds were formed, and on outcrossing 320 tetraploid hybrid flowers to *M. sativa* 3.3 seeds were formed, on the average, for each flower pollinated. Evidently the tetraploid hybrids have a degree of self-incompatibility intermediate between that of the two parental types. The difference in seed set obtained on intercrossing and outcrossing the tetraploid hybrids may be due in part to chromosomal irregularities.

Cytological examination of the tetraploid hybrids usually shows a regular behavior at meiosis. In the first division 16 bivalents (figure 28) are commonly formed. Lateral views of first metaphase plates, however, frequently show several univalents (figure 29). The univalents often divide during the first division and are commonly seen lagging between the two main groups of chromosomes during the second division (figure 30). The irregularities may be sufficient to lower appreciably the viability of at least the male gametophyte.

The tetraploid hybrids are fertile and pairing is usually normal. Since an unreduced set of 16 chromosomes has been received from the diploid *falcata* parent, these chromosomes probably form eight bivalents by autosyndesis. It is necessary to assume, therefore, that two sets of eight chromosomes have also been received from the *sativa* parent and that they too may pair by autosyndesis. The chromosome behavior in the tetraploid hybrids suggests that the *falcata* and *sativa* chromosomes are homologous and interpair freely.

Other crosses in Medicago were attempted between *M. lupulina*, *M. platycarpa*, *M. ruthenica* and another diploid ($n=8$) unidentified species on the one hand, and the diploid *M. falcata* and the tetraploid *M. sativa* on the other. The crosses were entirely unsuccessful.

DISCUSSION AND CONCLUSIONS

The difference in the ease with which hybrid seeds are obtained following reciprocal crosses between *Medicago sativa* ($n=16$) and *M. falcata*

(n=8) is similar to that occurring in Avena (KIHARA and NISHIYAMA 1932) and Triticum (WAKAKUWA 1934, BOYES and THOMPSON 1937) although, unlike the cereals, Medicago seeds do not have endosperm at maturity. Fertilization occurs, but is somewhat delayed when the female has the higher chromosome number. When the female has the lower chromosome number the rate of nuclear division in the endosperm seems to be out of balance with the synthesis of protoplasm and the formation of cells. When the female has the higher chromosome number, on the other hand, the nuclear divisions in both the embryo and endosperm are slower but cellular divisions in the endosperm occur early in both Avena and Triticum and some viable seeds are produced.

The quantitative chromosome balance of the three tissues, embryo, endosperm, and parental tissue, has been stressed in connection with ovule abortion (GAIRDNER 1926, MÜNTZING 1930). It has already been shown, however, that the chromosome number relation of the embryo to the endosperm cannot be the chief cause of the abortion (MÜNTZING 1933). The present paper now suggests, since the divergence from the normal balance is greatest in the case of the tetraploid Medicago hybrid embryos, that the relation of the chromosome number of the embryo and endosperm to that of the mother tissue is not the primary cause of the failure of development.

The genomic and chromosomal balance within the endosperm is considered the main reason for the reciprocal cross differences by WATKINS (1927), WAKAKUWA (1930) and THOMPSON (1930 a and b). This seems improbable, since in Medicago the genomes are closely similar, and the homologous chromosomes interpair freely. There are certain gene differences, however, and one or more of these may influence endosperm development sufficiently to cause the differences in reciprocal crosses in Medicago. The importance of a single specific gene in the success of a species cross has been demonstrated (HOLLINGSHEAD 1929).

In Medicago the tetraploids develop, whereas the triploids usually fail. An immunity reaction (KOSTOFF 1930), against the triploids only, seems an unlikely explanation. According to EAST's hypothesis (1935) the triploid hybrids should develop as readily on the diploid parent as do the tetraploids, since both carry the same chromosomes although in different proportions. This interpretation, therefore, is also inapplicable.

When the male has the higher chromosome number the balance seems to be shifted in favor of more rapid pollen tube growth and subsequent nuclear divisions. The rate of physiological activity and cell division in the endosperm seem correspondingly decreased. When the male has the lower chromosome number the nuclei of the embryo and endosperm are not stimulated (compare figure 12) to a sufficiently rapid rate of division

(KIHARA and NISHIYAMA 1932). The developing mother tissue utilizes the incoming food materials, causing the abortion of the ovule.

When an unreduced gamete functions in the diploid, however, the chromosome relation is proportional to that normally present in the parent plants and balanced rates of physiological activity occur. The primary reason for the differences arising in reciprocal crosses seems, therefore, to be the rate of physiological activity which is initiated in the embryo and endosperm at the time of fertilization, and this is dependent in large part on the chromosome number of the functioning gametes.

Hybrids having more chromosomes than normally expected have frequently been reported (BREMER 1923, CRANE and DARLINGTON 1927, GAIRDNER and DARLINGTON 1931, PETO 1934, SKALINSKA 1934, COLLINS and LONGLEY 1935). In each case, as in the tetraploid Medicago hybrids, it is assumed that due to the functioning of an unreduced female gamete the extra chromosomes have been received from the mother plant. Many examples have been reported in which fertilization has apparently been by an unreduced male gamete. Unreduced gametes are frequently formed by restitution in hybrids (LEDINGHAM and THOMPSON 1938).

Other possibilities of the origin of the tetraploid hybrids must be considered, since in Medicago it is not easy to prove that unreduced gametes are responsible. An integumentary cell extending into the embryo sac (COOPER 1935, Plate I, M, O) might have been fertilized, but all 643 developing embryos examined in this study arose in the normal micropylar position. Secondly, the eight female chromosomes might divide and form a restitution nucleus before the male chromosomes have divided. This could occur since the nuclei do not fuse completely before the first zygotic division, and one group may be a little ahead of the other in normal alfalfa material (COOPER 1935, Plate II, B–I). This explanation, however, seems improbable since in divisions, including seven in which accurate counts were made, the chromosomes were all at the same stage; and if restitutions occur some amphidiploids should form. Both these hypotheses explain how tetraploid embryos may be formed, but the endosperm remains the same and would probably cause the abortion of the tetraploid as well as the triploid embryos.

Triploids, and sometimes tetraploids, should arise in natural populations of the diploid *Medicago falcata* if unreduced gametes are formed. These probably occur in some plants (LARSEN and WESTERGAARD 1938) but the abnormal development of the endosperm, when the male and female gametes have different chromosome numbers, would prevent the occurrence of triploids in Medicago.

The diploid *Medicago falcata* may occur with the tetraploid *M. sativa* without intercrossing (KLINKOWSKI 1933). There are, however, no dis-

tinctive differences between the two species; and when triploid and tetraploid hybrids are formed, the homologous chromosomes interpair normally. The diploid form of *M. falcata* may approximate an original type of common alfalfa, and in it occasionally autotetraploids may still occur. *M. sativa* crosses freely with all tetraploid forms arising from *M. falcata*, giving a highly polymorphic group, with 32 somatic chromosomes, within which species differentiation is impossible. The diploid *M. falcata*, on the other hand, forms a homogeneous group which can be distinguished by chromosome number, size characters, the inability to cross with *M. sativa*, and by various gene differences, since certain *sativa* characters are unknown in the diploid *M. falcata*.

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SUMMARY

The diploid form of *Medicago falcata* does not cross readily with the common tetraploid *M. sativa*. When the former is the female, fertilization occurs, and development of the embryo proceeds slowly for about two weeks. The ovary continues to develop and forms a mature pod even though the ovules abort. When *M. sativa* is the female parent in the cross, fertilization is delayed and development of the endosperm and embryo usually stops after the second day.

The difference in seed development in reciprocal crosses seems to depend on the rate of physiological activity and cell division initiated in the endosperm at the time of fertilization. The chromosome number of the male and female nuclei is of considerable importance in this connection. There is some evidence that if the development of the endosperm and embryo is too slow some part of the ovule or ovary develops at their expense until abortion occurs.

Only two triploid hybrids were formed when *M. sativa* was the female parent, although the cross was repeated many times. Usually eight bivalents and eight univalents were present at first metaphase but some female gametes were viable in outcrosses to the parental types. The derivative plants are highly variable but the flower and pod characters and the chromosome number tend to be close to those of the recurrent parent.

When the cross was made in the other direction and the diploid *M. falcata* was the female parent only a few tetraploid hybrids were obtained. The flower color and pod shape are closer to the *falcata* type than in the

triploid hybrids for in the tetraploids twice the usual inheritance of the diploid *M. falcata* has been received. The fertility of the tetraploid hybrids is high, 16 bivalents usually forming, and the next generation is highly variable.

Homologous chromosomes of *M. falcata* and *M. sativa* interpair freely and there is no criterion by which the forms can be separated into distinct species. The diploid form of *M. falcata* does not cross readily with *M. sativa* due to faulty development after fertilization.

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SOMATIC MOSAICS IN THE DOMESTIC PIGEON

WILLARD F. HOLLANDER AND LEON J. COLE
University of Wisconsin, Madison, Wisconsin[1]

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MOSAIC or chimeric effects not of artificial origin may conveniently be divided into two classes, those which are frequent, and customarily associated with special genotypes, and those which are rare and unpredictable. Variegation and eversporting are examples of the former class. In the pigeon a condition known as "flecking" is of this sort. The other class includes gynandromorphs and chimeras generally.

These phenomena have received little attention in the pigeon. We shall present a review of the known facts, together with new cases which have come under our observation, and suggestions as to the possible causes involved.

FLECKING ASSOCIATED WITH SEX-LINKED FACTORS

In the wild type or "blue, black-barred" pigeon (*Columba livia*) no flecking has been observed. Flecking is found associated with three sex-linked color factors, which, as indicated later, are probably all alleles. Each of these is dominant to its wild-type allele, and each is responsible primarily for a particular kind of "bleaching" effect, that is, the plumage is lighter in color than that of the wild type. The apparent mutation, inactivation, or loss of such a factor in areas of irregular size and distribution in the feathers produces the flecking.

The most common of these factors is the "dominant red" of COLE and KELLEY (1919), or, as we shall call it, more specifically, "ash-red" (figure 1). HAWKINS (1931) demonstrated that the factor for sex-linked recessive "chocolate-brown" plumage color is a third allele at the ash-red locus. He symbolized these alleles, in descending order of dominance, B^A (ash-red), B (wild type), and b (chocolate-brown). HAWKINS reviewed the literature on flecking and reported new observations of his own. He found flecks only in heterozygous males, and suggested that flecking is merely a consequence of heterozygosity. Thus, males of the constitution B^AB showed only flecks of B phenotype, while males of the constitution B^Ab showed only flecks of the b phenotype; females, since they possess but one sex chromosome, cannot be heterozygous in the strict use of the term.

However, STEELE (1926) had definitely stated that he had observed "dunnish" flecks in dominant red females. We also have found flecking in many such females, and the flecks are invariably of the b type. HAWKINS'

[1] Papers from the Department of Genetics, Agricultural Experiment Station, No. 246.

suggestion that flecking is due to heterozygosity as such is therefore inadequate.

The sex difference in flecking was vaguely recognized for a long time by breeders (Cf. DARWIN, 1900, Vol. I, Chap. 5, p. 167), but the first clear outline was given by STEELE (1926). He stated that males more often exhibit flecking than do females; that flecked males generally show more flecks than do flecked females; and that flecks in females are not as dark in color as those in males. HAWKINS (1931) attempted to find whether an

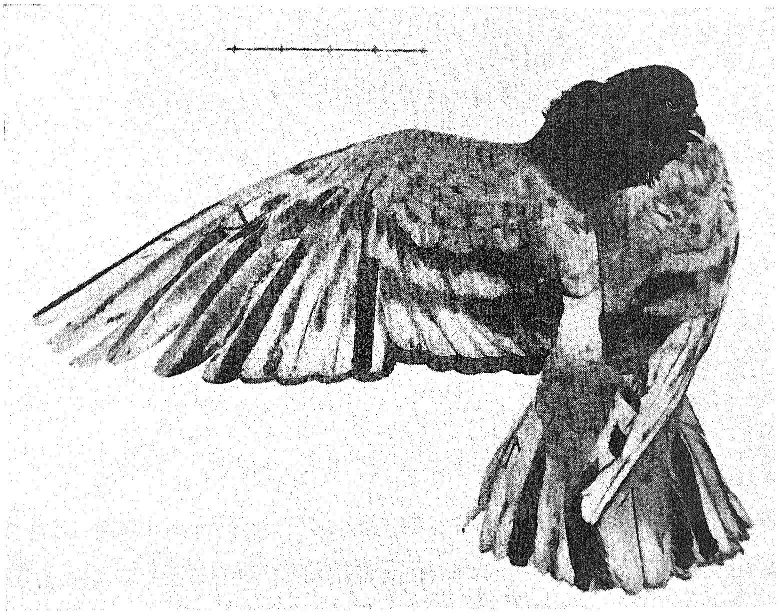

FIGURE 1.—Male D325Z, age $8\frac{1}{2}$ years, showing large amount of black flecking. Genotype: B^AB (ash-red).

endocrine differential is involved by castrating flecked males and observing subsequent feather regeneration. No change in fleck color or in general incidence of flecking was found.

Age has an important influence on flecking. The sex differences are already apparent in the juvenile plumage, but the flecking tends to increase in amount with each moult, so that, in old males particularly, it is extremely marked (figure 1).

The second most common factor associated with flecking is "almond," symbolized *St* by WRIEDT and CHRISTIE (1925). The color is basically yellowish-ashy to white, depending on associated factors. This factor is characteristic of the almond Tumbler, Oriental Roller, and Magnani Modena breeds. Flecking is abundant in both sexes, but as GHIGI (1908)

has shown, there is a marked sex-difference in quantity, as with ash-red. Unlike ash-red, the flecks in almond females are not chocolate in color, but show black (wild type) pigment. The flecking increases with each moult, as in the case of ash-red; old males may become more black than almond in appearance.

The third factor associated with flecking is "faded," *Of*, (HOLLANDER 1938). It has since been proved to be sex-linked. The general effect of this factor is much less obvious than almond or ash-red; the plumage is only slightly bleached. Flecking in faded birds is about the same in amount as that in heterozygous young ash-red males. It differs from ash-red and almond in that no sex difference or age difference has been apparent, but as in almond, the females have black pigmented flecks.

The relationships of all these three factors to one another are not yet fully determined but allelism is strongly indicated. Linkage tests of almond with another sex-linked factor, the recessive "dilution," *d*, were made by WRIEDT and CHRISTIE (1925); crossing over was around 50 percent. The amount of crossing over between ash-red and dilution was also very high in tests made by COLE and KELLEY (1919) and subsequent investigators. Since almond and ash-red both appear to lie at a distance from dilution, they probably are close neighbors of one another, if not actually allelic. FELDMAN (unpublished)[2] made a test of this possibility. Males heterozygous for ash-red and almond were produced; these resembled almond except that the flecking was of the ash-red color. Preliminary breeding tests with these males gave no crossing over.

We have obtained the male heterozygote of almond and chocolate. In this case a most unexpected result appeared: the flecks are chiefly chocolate, but a few are wild type. No linkage tests have yet been attempted. Faded has not yet been tested with almond or with chocolate, but it should be noted that FELDMAN obtained faded originally in the son of an almond ("Parlor Tumbler") female. By the rules of sex-linked inheritance, this son should have been almond; the best explanation so far advanced is that almond gave rise to faded by mutation. In preliminary linkage tests with dilution, faded has given a high rate of crossing over, while with ash-red it has given only non-crossovers. Males heterozygous for ash-red and faded resemble ash-red in general but their flecks are of the faded coloration.

In view of the above indications that almond and faded are situated at the B^A locus, we shall for purposes of discussion assume that all are alleles, and refer to them as the *B* series.

[2] The work referred to here was done by DR. H. W. FELDMAN at the University of Michigan He has generously given us valuable birds of the almond and faded types, as well as access to his records.

FLECKING ASSOCIATED WITH AN AUTOSOMAL FACTOR

Only one autosomal factor has been found accompanied by flecking. This factor is "grizzle" (G), also dominant to the wild type, and responsible for a whitening of the plumage. In a few heterozygotes we have observed large portions of feathers clearly lacking the grizzle factor, and therefore quite comparable to flecking associated with the sex-linked factors. It is difficult however to be certain that small areas lack grizzle. No further study of the flecking with this factor has been undertaken.

MUTATION IN THE SEX-LINKED B SERIES

This series may be symbolized provisionally in descending order of dominance, B^{St} (almond) $>B^A$ (ash-red) $>B^{Of}$ (faded) $>B$ (wild type) $>$ b (chocolate). The general effect of the genes dominant to wild type is to reduce the black pigmentation gradually to light gray or white in some areas and to red or yellow in others. These light colors serve as a contrasting background for dark flecks of a more recessive color. On the hypothesis that the dominant gene is completely lost or inactivated in the area of the fleck, the color of the fleck should be governed by the remaining allele. Thus if the gene B^A were eliminated in the heterozygote $B^A\ B$ the fleck pigmentation should be wild type, while in the heterozygote $B^A\ b$ the fleck pigmentation should be chocolate. In females of any of the three dominant types chocolate flecks (the "residual" condition) would be expected on the assumption that b is the lowest possible allele at this locus, but such is not the case for almond or faded. Furthermore, the male almond-chocolate heterozygote, $B^{St}\ b$, has not only flecks of the expected chocolate pigmentation but also some of wild type. The conclusion seems unavoidable that there is not actual loss or complete inactivation of the dominant allele in all cases. This also precludes an explanation based on the loss of an entire chromosome and further evidence against such loss is found in males heterozygous for both the ash-red and the dilution loci of the sex chromosome: $B^A\ D/B\ d$. The flecks in such males were always black. If the whole chromosome containing B^A were lost also in some cases, the flecks should then show the dilution phenotype, provided that this factor can produce its effect autonomously in development. That it can so act is indicated later in connection with chimeras.

A more plausible genetic basis for the flecking seems to be offered by the hypothesis of somatic mutation of labile genes. Such genes have been shown to exist in a number of species of animals and plants. In this case it is only necessary to assume that the dominant genes tend to mutate to alleles lower in the series, but not always to the same one even in a single bird. Presumably these mutations occur in homozygotes equally as often as in heterozygotes, but no visible result would be expected unless both

genes in the cell mutated. Similarly, in males heterozygous for two of the dominants, mutation of the lower allele would never be observable unless by coincidence the higher gene mutated also. There is no evidence that either the wild type or chocolate alleles are at all labile; apparently mutation is a characteristic of the genes dominant to wild type and the higher the allele in the series, the more labile it is. Since almond heterozygotes show such a large amount of flecking, we would not be surprised if almond homozygotes should show a few flecks; the mutation rate seems so high that both genes might well occasionally mutate in the same cell. However, no observations on mature male almond homozygotes have yet been made.

In table 1 is summarized present knowledge of the *B* series genotypes and phenotypes, with notes on the flecking.

FLECKING IN OTHER BIRDS

The pigeon is not alone among birds in exhibiting flecking. Essentially the same phenomenon, under names such as "fault feathering" or "exceptional feathers," is found in the "blue" domestic duck, the slate turkey, and several sorts of chickens, such as Barred Plymouth Rocks, Andalusians, and dominant whites. In certain of these forms it has received considerable attention both from breeders and from investigators, and has been recognized as a special phase of the problem of coloration because of the high degree of irregularity. In all these species, as in the pigeon, the genetic factors involved are more or less dominant to the wild type; furthermore, the flecking does not occur in homozygotes, except in the white Andalusian fowl. The Andalusian, seeming exceptional, requires special discussion. SEREBROVKSY (1926) states that flecking in the heterozygous ("blue") Andalusians is always black (wild type allele), while it is "blue" in homozygous whites, and much more striking than in heterozygotes. He explains these facts by the assumption that "loss" of one allele occurs. The homozygote having two labile alleles, blue flecking should be twice as abundant as in heterozygotes. We have examined a number of Andalusians, and find SEREBROVSKY'S treatment satisfactory. We may add that within large blue flecks (in homozygotes) black flecks are occasionally to be found. These would indicate "loss" of both alleles. The reason for the peculiar status of the Andalusians seems simply the fact that the factor involved is only partially dominant to the wild type allele, the heterozygous condition being phenotypically distinct.

The Andalusian factor and dominant white in fowls are not sex-linked, and apparently sex-linkage is not involved in the duck or turkey. But most of the investigation of the flecking has centered around two independent dominant sex-linked color factors, "bar" and "silver," in the chicken. SEREBROVKSY (1926) and HERTWIG and RITTERSHAUS (1929)

TABLE I

Genotypes and Phenotypes of the B Series.

GENOTYPE	BASIC COLOR	FLECKS	EXPECTATION ON BASIS OF LOSS
		Almond Genotypes	
Males			
$B^{St}\ B^{St}$	*	*	Creamy; no flecks
$B^{St}\ B^{A}$	Creamy	Ash-red	As found
$B^{St}\ B^{Of}$	*	*	Creamy; faded flecks
$B^{St}\ B$	Creamy	Black	As found
$B^{St}\ b$	Creamy	Chocolate and black	Only chocolate flecks
Females			
$B^{St}-$	Creamy	Black	Chocolate flecks
		Ash-red Series	
Males			
$B^{A}\ B^{A}$	Ash-red	None	As found
$B^{A}\ B^{Of}$	Ash-red	Faded	As found
$B^{A}\ B$	Ash-red	Black	As found
$B^{A}\ b$	Ash-red	Chocolate	As found
Females			
$B^{A}-$	Ash-red	Chocolate	As found
		Faded Series	
Males			
$B^{Of}\ B^{Of}$	*	*	Faded, no flecks
$B^{Of}\ B$	Faded	Black	As found
$B^{Of}\ b$	*	*	Faded; chocolate flecks
Females			
B^{Of}	Faded	Black	Faded; chocolate flecks
		Black Series	
Males			
$B\ B$	Black	None	No flecks
$B\ b$	Black	None	Chocolate flecks if mutation occurred
Females			
$B-$	Black	None	
		Chocolate	
Males			
$b\ b$	Chocolate	None	As found
Females			
$b-$	Chocolate	None	As found

* Not studied or insufficient information.

observed the frequencies of each kind of fleck in Barred Plymouth Rocks and crosses possessing both these factors, and in each sex. SEREBROVSKY concluded that segregation of whole sex chromosomes, with or without crossing over, would adequately explain the facts, while the latter authors decided that only part of the chromosome—usually the part containing

the bar factor—was lost. In either case, a genetic change in the soma appeared to account for the exceptional feathers.

HERTWIG and RITTERSHAUS gave as further evidence of a genetic change that, after plucking, the fault feathers are replaced by new ones of the same type. JUHN (1933) has demonstrated however that this result is not regularly found. She concludes that her findings "do not support the genetic interpretations advanced," which, she adds, "are untenable from an embryological point of view," though she does not specify in what way. Her explanation of the exceptional feathers is that a physiological threshold allows the recessive factors in the birds' make-up to function at times, depending on "the interaction of the genetic factors . . . with variable morphogenetic factors such as rate of growth . . . " In other words, a physiological control of the action of these genes is postulated, the dominant being expressed under certain local internal conditions, and suppressed under others.

MONTALENTI (1934) has also investigated feather succession in Barred Plymouth Rocks. He found that follicles from which "abnormal" feathers are plucked always regenerated abnormal feathers, though in most cases the successive feathers differed considerably. If the feathers were completely abnormal (black, in these chickens) they were generally followed by new ones of exactly the same sort. MONTALENTI sums up his observations and deductions as follows: "It appears . . . that the *range of action of the genes for the barring* in mosaic feathers may vary considerably in successive generations. Sometimes the barring does not appear at all in some of them, although this factor is potentially present in the follicles concerned, as it is proved by its manifestation in successive generations of feathers." (Italics ours.)

THE DEVELOPMENTAL BASIS OF FLECKING

We have concluded so far, on the basis of fleck coloration, that flecking in pigeons is the result of genetic change in the soma, and that this change is probably mutational in nature. We must next examine the facts of feather development to determine whether this conclusion can be accepted.

We have observed successive feathers from a large number of follicles in different specimens of the *B* series. As in chickens, there is a great deal of variability in the successive feathers; nevertheless, where the fleck is so large as to extend from the tip to the base of the feather, and especially if an entire vane or more is involved, repetition in successive feathers is obvious (figure 2). The smaller the flecks the less tendency there is to repeat in detail, but the same absolute variability would be expected to produce more apparent alteration of the small flecks than of the large

ones. At any rate, we may conclude that in the case of the great flecks, a relatively permanent differentiation exists in the feather primordium with respect to potentialities for color production.

If we interpret LILLIE and JUHN's latest (1938) contribution correctly, they now consider growth of the feather to be mainly axial, rather than concrescent as earlier assumed. Any genetic difference existing in the "collar," such as might occur from mutation, should therefore result in a

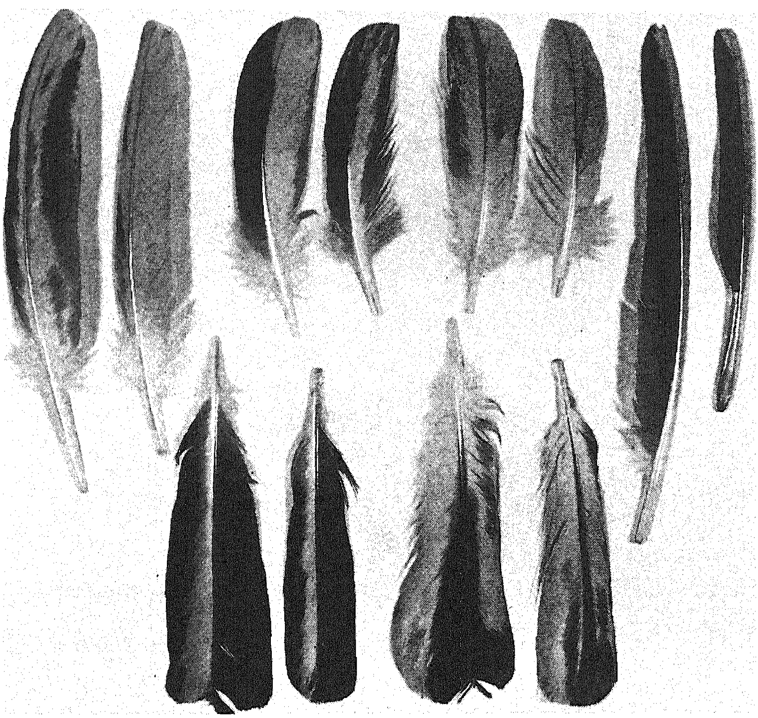

FIGURE 2.—Pairs of successive feathers from six follicles from aged flecked male, 2515E (genotype B^A B). In each pair, the younger feather is to the right of the older. Above, wing feathers; below, tail feathers. The first feathers were all pulled the same day, and the successors a month later. Note that the more outstanding fleck areas (black) tend to be repeated, with fair fidelity in most cases, and that the fleck areas in general run longitudinally.

corresponding longitudinal streak in the completed feather. This condition is often approximated by the larger flecks, and the structural difference shown in figure 15 gives striking evidence of definite axial growth. The small flecks appear as irregular islands. The irregularities in shape and size, as well as in replacement, may be accounted for by one or more of several processes: 1. New mutations which would affect very small areas may be occurring intermittently in the active part of the collar. 2. Irregularities of outline and lack of continuity, producing flecks rather than continuous streaks, may result from uneven relative growth of different

derivative cells in the collar. 3. As DANFORTH (1939) has emphasized, the early precursors of the pigment cells are apparently capable of migration; furthermore, these specialized cells are large and have long branching processes which deposit pigment some distance from the original position of the cell (GREITE 1934).

That physiological differences and changes may have an influence on the incidence of mutation and possibly on the relative growth of the mutated portions is as yet undeniable. Sex and age differences in the quantity of flecking, as described in connection with ash-red and almond, point to some measure of metabolic control. Subsidiary genetic factors, subject to selection, also may have an influence on the degree of flecking, as is shown by differences in specimens physiologically comparable.

CHIMERAS

Gynandromorphism is a well-known type of chimera in domestic fowls, according to CREW and MUNRO (1938), but no case has been reported in pigeons. The lack may be due to the difficulty in recognizing sexually abnormal pigeons; external differences between male and female are so slight that a gynandromorph might easily be overlooked.

Chimeras of feather color and structure have, however, occasionally been observed. The flecking and chimera types have much in common but chimeras differ from the flecking type in being relatively rare and unpredictable in occurrence, and in having larger areas affected, including whole groups of feathers, and even considerable portions of the body. For convenience, and without any necessary implication as to cause, these will be referred to as the "mutant areas." They are, in general, though not always, similar to flecking in being attributable to some sort of loss of the dominant allele in a heterozygote. To account for the large areas involved, it must be assumed that the "mutation" occurred earlier in ontogeny than is the case with flecking, possibly in some cases even as early as the first cleavage division.

For convenience of treatment, the pigeon chimeras which have come to our attention (26 in all) are classified below according to the principal factors involved.

1. *Chimeras involving ash-red* (B^A).

LYELL (1877, p. 48) states that he once bred a "mealy with black shoulders." It is clear from his other descriptions that by "mealy" LYELL refers to the ash-red condition, while the mutant area is of the *B* phenotype. He did not give sex or pedigree of the specimen.

Two somewhat similar male mosaics have been studied at this laboratory. The first, male 2067B, was a crossbred Tumbler produced by an

ash-red male and a black female. Most of the plumage of this specimen was typically ash-red with black flecks, but on the crop, back of the head, and inter-scapular region were large patches of black feathers. These patches remained during the six years of the bird's life. A progeny test indicated that his constitution was $B^A B$, as was to be expected. The second mosaic, 2411A, exhibited much more extensive B areas (figure 3).

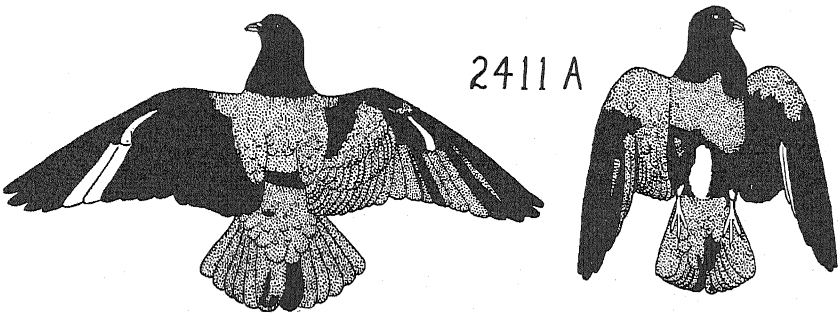

FIGURE 3.—Diagrams of male chimera 2411A. In these and following diagrams, black represents the dominant allele, stippling represents the recessive allele, and white represents white-spotting. Dominant allele here, B^A (ash-red); recessive allele, B (blue checker).

This bird was a Homing pigeon obtained from a Milwaukee, Wisconsin, breeder; the parents were said to be an ash-red male and a wild-type-colored female. The mosaic was tested with a wild type female; of the five offspring obtained, two were typical ash-red, and three showed wild-type pigmentation. Thus the gonads were, at least in part, of the constitution $B^A B$.

LEVI and HOLLANDER (1939) report two additional cases, with illustrations. Both were $B^A B$ males, by pedigree and, in the one case tested, by progeny test. Both showed a large amount of B plumage, and both possessed white-spotting, extensive in one. A most unusual feature of one of these mosaics was the change, in the transition from juvenile to adult plumage, of a large part of the B plumage in the tail back to the proper color, ash-red. Such reversion has not been observed in any other chimera.

A male bird (E284E) of genotype $B^A B$ had relatively few and small flecks in its juvenal plumage. Now, in its first adult plumage, it has abundant flecks and by the time it is several years old it will presumably be fully as flecked as D325Z, shown in figure 1. In addition, E284E has on the left side of its head a black patch which extends from near the base of the beak, beneath the eye to the occiput (figure 4). The total area of this patch is less than might occur on a single tail feather; it differs from the latter, however, in that it involves a good many contiguous feathers instead of a single one, and its extent has not increased with the molt.

This specimen may accordingly be included as a chimera.

In the above six cases, the mosaicism can be accounted for by the hypothesis that the B^A factor, in heterozygotes, has mutated to B (or b) or become lost or otherwise inactivated, as in the case of flecking, but at some time early in ontogeny.

A more complicated problem is presented by male 2721E, bred at this laboratory. In addition to the sex-linked ash-red factor, an autosomal feather pattern factor is involved. The bird's father was a dun of the T

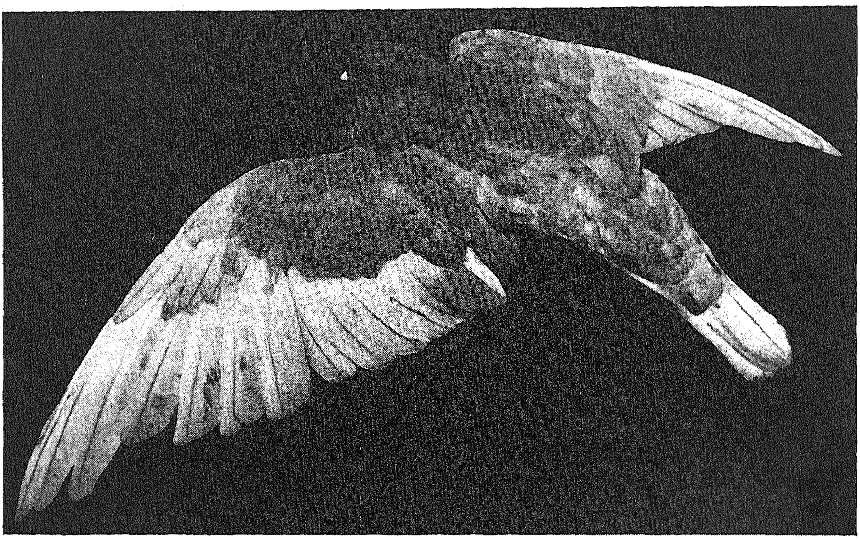

FIGURE 4.—Young ash-red male E284E (genotype B^A B) showing black flecks on individual feathers and a mutated patch of black feathers on the head. The latter presumably represent a single early mutation, making this bird, according to definition, a chimera.

pattern, genotypically $B\ d/B\ d;\ C^T/C$. The mother was a typical ash-red, of the constitution $B^A D/-;\ c/c$. The mosaic is ash-red in the checker pattern, with flecking, and is therefore genotypically $B\ ?/B^A\ D;\ C/c$. On the right wing there is a large patch of brownish feathers (enclosed by a black line, figure 5) whose color we have not been able to identify with certainty. The feathers of this area have a few flecks, which are black as in other parts of plumage. Furthermore, the feathers appear to be of the T pattern, rather than checker. Possibly the brownish color arose by recessive mutation of B^A to some new allele in the series, but a simultaneous mutation of C to C^T in another chromosome seems highly improbable. No very satisfactory explanation of this mosaic has been deduced. Polyspermy might perhaps be invoked but this would involve other complications. No breeding tests were made.

2. *Chimeras involving chocolate* (b).

A male mosaic showing irregular areas of B and b plumage coloration, together with a good deal of white spotting, is reported and figured by LEVI and HOLLANDER (1939), in the King breed. By pedigree, the bird's genotype was Bb. This case would be most simply explained by the loss of B in certain areas. This is the only case we have of the mutation of B.

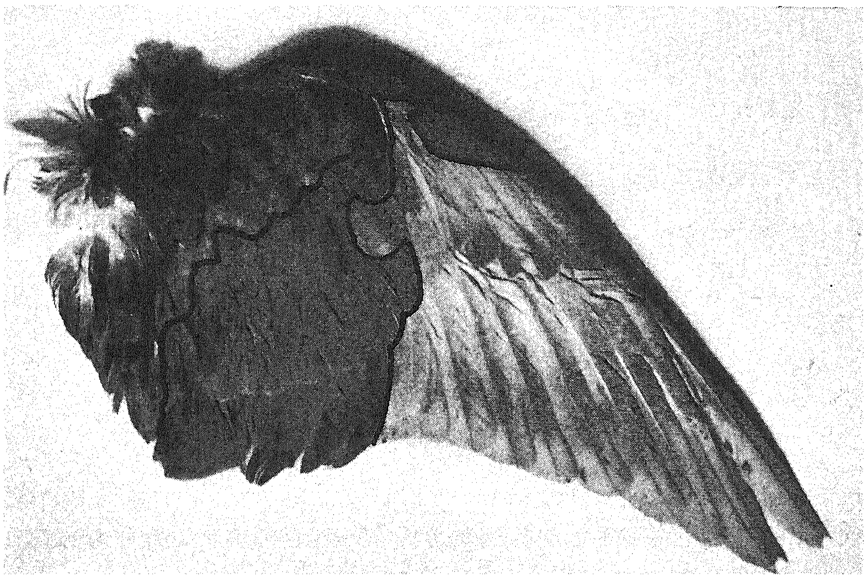

FIGURE 5.—Right wing from male chimera 2721E. The island of unusual brownish feathers is outlined by a black ink line. The surrounding feathers are ash-red (B^A B) with a few black flecks; a single black fleck also occurs in a lesser secondary covert in the island.

3. *Chimeras involving dilution* (d)

Two mosaics of dilution and its normal allele are known, both in the Carneau breed. KEESLING (1924) described a specimen whose father was yellow (dilution with autosomal recessive red: dd ee) and whose mother was red (D- ee). It was "a cock of beautiful type and good size; has yellow head with small red spots, red breast and neck, yellow wings and back, red wing flights and tail. The markings are sharply defined." From this description it seems that a high degree of symmetry existed. The constitution of the bird is, by pedigree, Dd, so that the appearance of d areas is unexpected, but may be accounted for by mutation of D to d.

A quite similar case, though not symmetrically marked, was observed by one of us (W. F. H.) at the Middleton Squab Farm of Norristown, Pa. This bird also was a male, apparently breeding normally. There was a

moderate amount of white spotting in the plumage. Nothing further is known about this bird.

4. *Chimeras involving grizzle* (*G*)

A single grizzle mosaic female crossbred Homer-Carneau, *Gg* by pedigree, is reported by LEVI and HOLLANDER (1939). Most of the bird is grizzle but the factor appears to be lacking on most or all of the left wing. There was a moderate amount of white spotting.

5. *Chimeras involving recessive red* (*e*)

a. *With recessive mutant areas.* METZELAAR (1926, p. 34) mentioned a recessive red mosaic. In correspondence of January 24, 1925, he described it in detail: "Crossing a pure recessive red Carneau with a brown silver King, a female young was obtained which shows both parental colors in a piebald form. The flights are pure red, so is the neck; the rest of the body is brown-barred but for a few flecks in the areas with clumped pigment. These flecks are red; not clumped but spread pigment. They are irregular islands of spread pigment within the clumped brown region." The brown in this mating is sex-linked chocolate, and is to be expected in female offspring. The red, however, is unexpected, but may be accounted for by assuming mutation of E to e in a bird of the composition $b/-$; E/e. Here again is a case with (apparently) a fair symmetry. The bird has been mounted, and is at present in the Museum of Zoology at the University of Michigan.

Two other mosaics involving recessive red were given us in 1934 by DR. H. W. FELDMAN from the University of Michigan colony. These were male sibs, 2708.1 and 2708.2, F_2's from recessive red grizzle white-spotted Tippler × black-laced Blondinette. Both were black with some grizzling and white-spotting apparent; in one, scattered patches of red feathers occur over the neck, crop, and scapular regions; in the other, only a few scapulars are red. Although neither specimen was progeny-tested, their genotype was probably Ee, and the red areas may be accounted for simply by loss of the normal allele.

Two similar cases have appeared at this laboratory. A female, 1158H, whose skin in juvenile plumage was preserved, closely resembles the above males but no entirely red feathers are present; instead, there are large red segments of the interscapular feathers and elsewhere. She also had the typical juvenile reddish edging on most other feathers. She was heterozygous for *e*, as the father was recessive red and the mother black. The remaining case is a male, 2688H (figure 6), which has a rather large area of recessive red color, and also a moderate degree of white spotting. No breeding tests were made, but as both parents were heterozygous for *e*,

this specimen in all probability is also. Here again the appearance of red may be attributed to loss of the normal allele.

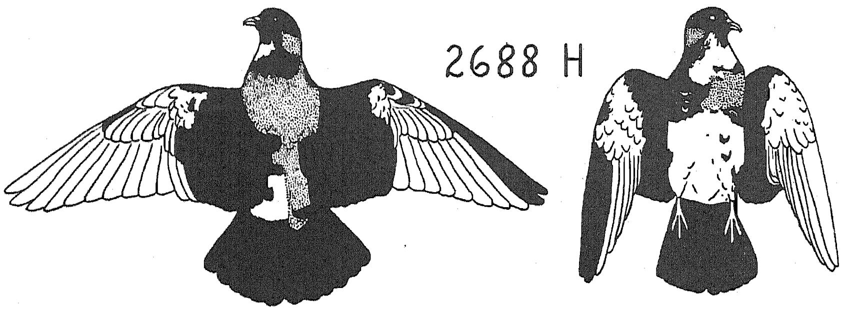

FIGURE 6.—Diagrams of male chimera 2688H. Black areas represent wild type allele (blue checker); stippled areas recessive red.

b. *With dominant feathers.* HORLACHER (1930, p. 341) mentions four recessive red specimens with one or more black feathers. Here we have

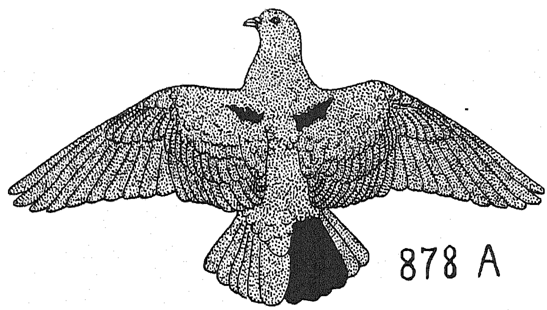

FIGURE 7.—Diagram of male chimera 878A. Stippling, recessive red; black, wild type allele (black).

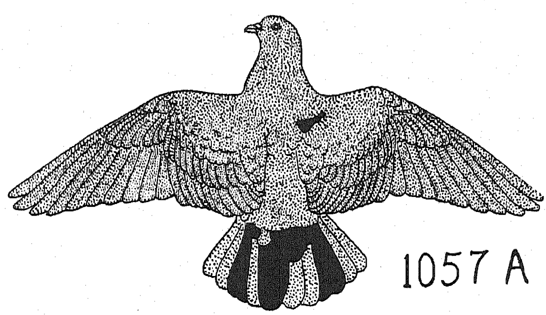

FIGURE 8.—Diagram of female chimera 1057A. Key same as in figure 7.

what appears to be a different sort of chimera from those treated above. Further information is available for two of these. One was a male, 1875C. Both parents were recessive red, and the first description of the specimen

(six years before HORLACHER's description) made no mention of any black feathers; HORLACHER found a single black wing covert in this bird. The other mosaic, 1057A, was a female (figure 8). This specimen has been

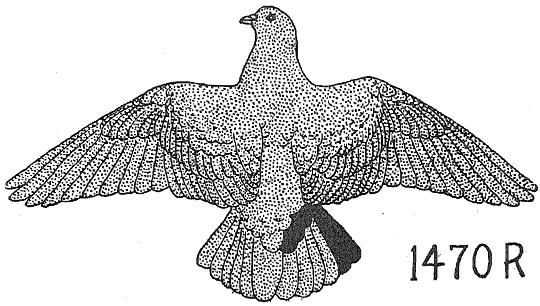

FIGURE 9.—Diagram of female chimera 1470R. Key same as in figure 7.

preserved; there are black feathers in the interscapular region and in the tail. No progeny test was made.

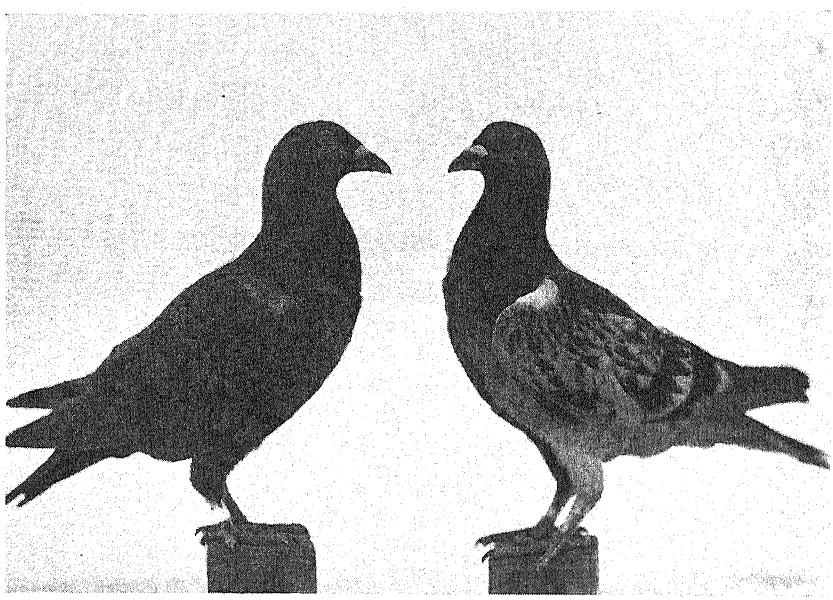

FIGURE 10.—Right and left aspects of "half-sider" *S* mosaic. Right side spread black, *S*; left side blue checker, *S*.

Two additional examples very similar to 1057A have been produced in the Wisconsin colony. One was a male, 878A (figure 7), and the other a female, 1470R (figure 9). These three mosaics are somewhat related to one another, the females being first cousins. Furthermore, a paternal uncle of the females, 925B, was described as recessive red with two black

coverts in the right wing. The parentage of all the above individuals was *Ee* male×*ee* female. It is therefore not possible to be certain of the genotype of the mosaics except by breeding test. The male 878A, mated with a recessive red, produced a total of three young, all typical *ee*. The female 1470R, in several matings with *ee* males, including one of her sons, produced 35 offspring, all typical *ee*, with no tendency to mosaicism. Apparently the genotype of these mosaics is therefore *ee*.

The only explanation which seems likely for this sort of mosaic is dominant mutation, but polyspermy could possibly, though very doubtfully, be invoked.

6. *Chimeras involving S* (*"spread black"*)

A male Homing pigeon (sex?) black on the right side and blue checker on the left (figures 10 and 11) is now at the Whitman laboratory of the University of Chicago. It was bred by MR. CALDWELL MARTIN of Denver,

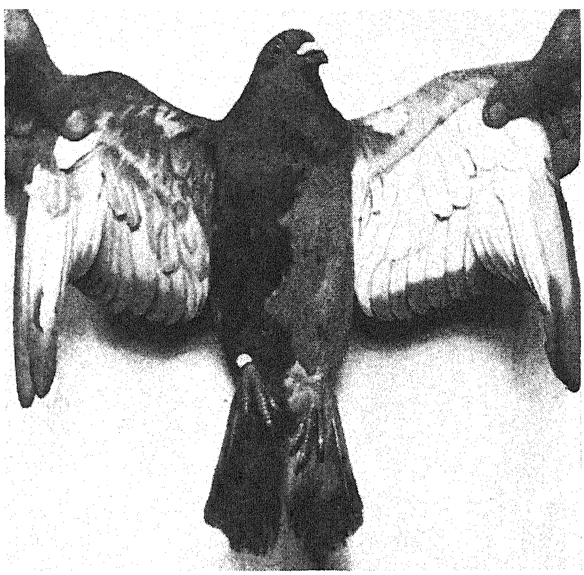

FIGURE 11.—Ventral aspect of "half-sider" *S* mosaic.

Colorado, and sent to DR. L. V. DOMM through the interest of MR. W. VAN RIPER of Denver. Apparently, the *S* factor, which produces "spread" blackness of plumage, is involved; both parents were black, but in another mating the sire proved heterozygous. It may be assumed that the initial constitution of the mosaic was *Ss*. Near the median division line some feathers exhibit both black and blue in longitudinal stripes (figure 12); this is apparently the result of genetically mixed tissue in the same feather follicle, since new feathers from these follicles have been very similar to the previous ones.

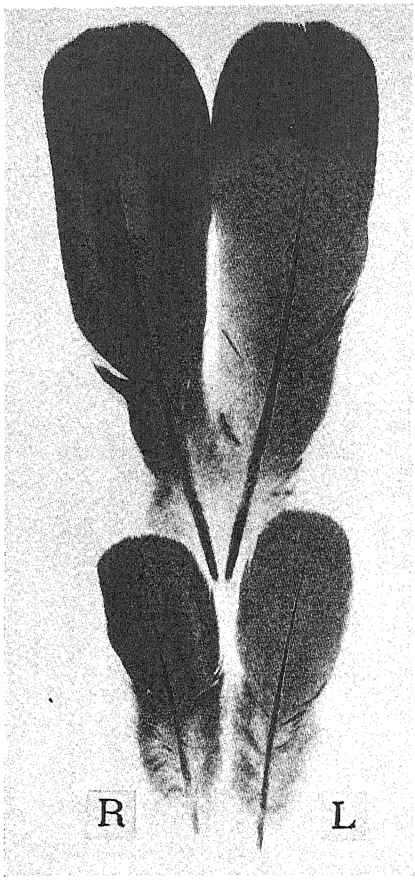

FIGURE 12.—Rectrices and dorsal major tail coverts from right and left sides of midline of "half-sider" *S* mosaic. Note the smoothly demarcated stripes on the feathers from the right side.

At the Wisconsin colony a more complicated mosaic involving *S* appeared. This specimen, D935K (figure 13), was killed while yet in juvenile plumage and before its interest had been fully recognized.

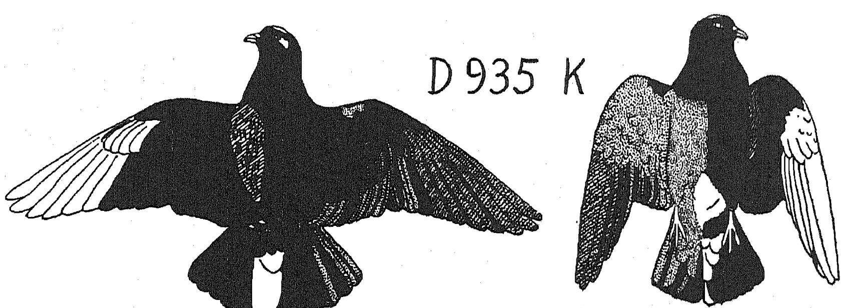

FIGURE 13.—Diagrams of female multiple mosaic, D935K. Black areas, black; stippling, blue with structural defect; shading, faded (?).

It was quite healthy and vigorous, but its flight was lopsided. Autopsy revealed a normal ovary. Only the wings and tail were preserved after description had been completed. Later, an X-ray photograph (figure 14)

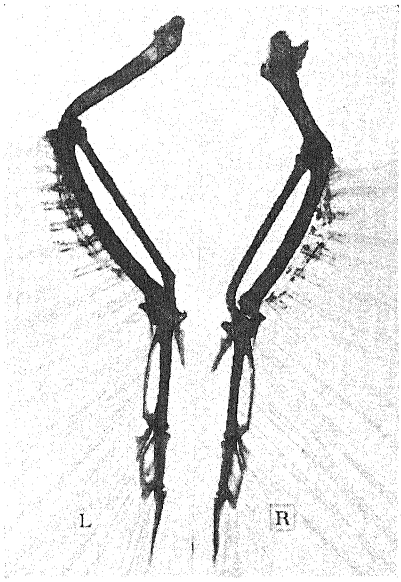

FIGURE 14.—X-ray photograph of left and right wings of multiple mosaic female D935K.

showed that the skeleton of the right wing was nearly 2 cm shorter than that of the left. Comparison of wing bone measurements of left and right wings and with a normal bird gave the following results:

	Normal bird	Mutant female, D935K	
		Left	Right
Humerus	46 mm	45 mm	35 mm
Ulna	53 mm	54 mm	48 mm
Digit (to last joint)	54 mm	49 mm	48 mm
Totals	153 mm	148 mm	131 mm

The feathers also of the right wing were shorter than those of the left; for example, the third left primary measured 183 mm, while the third right measured 170 mm; and the third left rectrix measured 130 mm, while the third right measured 125 mm. Furthermore, the feathers of the right side, and almost entirely in the regions of *s* coloration, were defective in structure, webbing rather poorly.

The color distribution of the bird is shown in figure 13. White spotting occurs only on the left side, which otherwise is spread black, except for the scapulars; these appear to be "faded." On the right side most of the greater feathers show longitudinal blue stripes of defective structure; often there are stripes of black with normal structure (figure 15). The

first two primaries are possibly of the "faded" color, and are also defective in structure.

The degree of asymmetry of white spotting in this specimen is very unusual and probably significant. None of the numerous sibs produced in the mating even approached this degree of asymmetry nor has it been observed in a great number of birds of similar type.

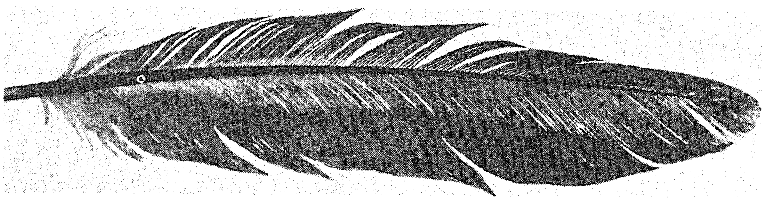

FIGURE 15.—A primary wing feather from the right wing of D935K. Note the even edge of the longitudinal structural fault zone.

The parents were healthy and quite normal; they were breeding regularly and excellently throughout the season when D935K appeared. The father was a common pigeon with a little white spotting and heterozygous for the sex-linked dominant factor *Of*, "faded." The mother was black (heterozygous for *S*), with a little white spotting; she was by pedigree 1/32 *Columba guinea*, but no remaining traces of this species were apparent, and fertility was quite normal. Further investigation of the ancestry has revealed no source for the structural anomalies in this mosaic. It is, however, quite obvious that this case cannot be explained by simple point mutation. Undoubtedly, more extensive chromosomal aberration is involved.

7. *Involving Silky* (*L*)

SPRUIJT (1931) described a Dutch Cropper pigeon with silky plumage, except for a few normal feathers in the tail and on the legs. Silky plumage is otherwise unknown in this breed. The specimen was mated with a sister, but produced only normal young. The possibility that a somatic mutation to the dominant silky factor, *L*, occurred is strengthened by the fact that a silky individual arising from normal parents presumably by germinal mutation, has been observed (COLE and HOLLANDER 1939).

THE GENETIC BASIS FOR CHIMERISM

No single explanation appears capable of accounting for all the chimeras. In other birds, such as chickens, budgerigars and finches, chimeras of fundamentally differing genetic basis have been reported by CREW and MUNRO (1938). Often simple loss of some kind in heterozygotes is a sufficient explanation. In other cases, non-disjunction has been postulated,

as for example in gynandromorphism or in birds not genetically heterozygous, or where size or other changes are involved. Somatic segregation was suggested by ASMUNDSON (1938) to explain a chimeric turkey. Such an explanation could possibly apply to some of the pigeon chimeras. Dominant mutation or polyspermic effects have not been suggested in other birds; WRIGHT and EATON (1926) found a dominant somatic mutation in a guinea pig, and polyspermy has been found to occur in *Drosophila pseudoobscura* by CREW and LAMY (1939).

TABLE 2

Summary of Chimeras.

		Mutant Area
1. Involving ash-red (B^A)		
LYELL (1887)	?	Simple recessive, B^A to B
2067B	♂	Simple recessive, B^A to B
2411A	♂	Simple recessive, B^A to B
LEVI AND HOLLANDER	♂	Simple recessive, B^A to B
LEVI AND HOLLANDER	♂	Simple recessive, B^A to B
E284E	♂	Simple recessive, B^A to B
2721E	♂	Complex; 2 factors involved
2. Involving chocolate (b)		
LEVI AND HOLLANDER	♂	Simple recessive, B to b
3. Involving dilution (d)		
KEESLING (1924)	♂	Simple recessive, D to d
HOLLANDER	♂	Simple recessive, D to d
4. Involving grizzle (G)		
LEVI AND HOLLANDER	♀	Simple recessive, G to g
5. Involving recessive red (e)		
(a) with recessive mutant areas		
METZELAAR (1926)	♀	Simple recessive, E to e
2708.1	♂	Simple recessive, E to e
2708.2	♂	Simple recessive, E to e
1158H	♀	Simple recessive, E to e
2688H	♂	Simple recessive, E to e
(b) with dominant feathers		
1875C	♂	Dominant, e to E
1057A	♀	Dominant, e to E
T49D	♂	Dominant, e to E
T55C	♂	Dominant, e to E
878A	♂	Dominant, e to E
1470R	♀	Dominant, e to E
925B	♂	Dominant, e to E
6. Involving S ("spread black")		
Chicago "half-sider"	?	Simple recessive, S to s
D935K	♀	Complex
7. Involving silky (L)		
SPRUIJT (1931)	♂	Dominant, l to L

For purposes of ready reference the chimeras described in this paper are summarized in table 2.

Simple recessive mutation, without the necessity of invoking chromoso-

mal loss, somatic segregation or other aberrancy, will explain the majority of the pigeon chimeras; mutation from recessive to dominant will explain all the others except 2721E and D935K, which are more complex. The fact that all the chimeras involving sex-linked characters are males may indicate that the explanation is really not so simple. Moreover, factors are involved which are ordinarily stable (B and D) as well as one (B^A) which, according to the flecking results, is regularly labile.

Polyspermy might possibly account for 2721E (group 1) and the reds with small black areas (group 5, b) but the results of polyspermy should be detectable in almost any mating of heterozygote with recessive homozygote and hence it cannot be very prevalent. Evidence for it has not been found in hundreds of suitable matings. Furthermore, there appeared to be some tendency for the black-on-red condition to occur in related birds. In this respect it differs from the other chimeras.

D935K (group 6) is the only known chimera in pigeons involving body structural characters and, in this case, feather structure as well. Apparently a single loss can account for this case only if S and white spotting, and presumably factors for structural development as well, are assumed to be on the same chromsome. There is no independent evidence to support this assumption. It would appear likely, however, that in this case a whole autosome, or more, was eliminated early in the development of the embryo.

The somatic nature of all the above pigeon chimeras is evidenced by failure of the condition to be transmitted in all cases when breeding tests were made. The tests were not always conclusive, it is true, but none gave any indication that the gonads were affected, even when the major portion of the body appeared "mutated," as in 2411A (see figure 3).

White spotting was present in at least 10 of the 26 chimeras described but probably as an incidental association. There are many essentially symmetrical patterns of white spotting in pigeons which breed more or less true. Although the developmental and hereditary basis for white spotting has not yet been successfully analyzed, it seems safe to conclude that it is not chimerism of the above sort because of its relatively predictable inheritance. Such marked asymmetry of the white as occurred in D935K is altogether unusual and is undoubtedly part of the mosaic pattern in this bird. This is the only specimen of this type that has come to our attention, but possibly the "scherzo" pigeons mentioned by Italian writers, as quoted by HOLMES (1921), are of this sort.

THE DEVELOPMENTAL BASIS OF CHIMERISM

No obvious law has been found governing the distribution of the differing areas in chimeras. In other species of birds CREW and MUNRO (1938)

found that sharp left-right asymmetry was common, but in pigeons we have found only one such case. Blotchy effects are most common and to a certain extent comparable cases may be cited in such forms as guinea pigs and Drosophila. In some cases the mutant areas are of more or less central location (for example, 2688H, group 5, and 2411A, group 1); in METZELAAR'S mosaic (group 5) they are distal. Only the nape of the neck and the interscapular region seem to be affected with any considerable regularity.

The interpretation of these heterogeneous arrangements in terms of development is not easy, especially as cell lineage in the definitive differentiation is obscure. Also, in considering color, the peculiar behavior of the pigment cells must be considered. Studies in this field, as summarized by DANFORTH (1939), indicate that in the embryo the "pigmentoblasts" have neural origin and migrate to their final positions. If the migration is irregular it may help to explain the irregularity of chimerism. At any rate, several discontinuous areas are presumably not to be interpreted as due to separate mutations. It would be difficult to explain why, considering the rarity of chimeras, a bird which had one mutation in its early development should have several simultaneous ones.

Unlike flecking, the color arrangements in chimeras have proved permanent through successive molts except in one case (reported by LEVI and HOLLANDER, group 1). In this case part of the mutated feathers in the tail changed in a reverse direction, back to the dominant color. We have no explanation of this case, but until more is known concerning color determination we may assume that it might harmonize with genetic interpretation.

In the *S* mosaics (group 6) clear striping effects, both of color and structure, were noted. The striping is more clean-cut than in flecking, and fits excellently the theory of axial cell-lineage in the feather. The *S* factor affects not pigment quality, but the arrangement or pattern of the pigment in the cell, and this may well be determined by the tissues in which the pigment cells reside rather than by the pigment cells themselves. The tissue cells having no tendency to wander in the growing feather, mosaicism would be expected to show up as sharply distinguished axial striping, and the same is true of a structural difference.

This study of chimeras in pigeons demonstrates that the factors involved are autonomous in development, that is, two different alleles are able to express themselves clearly in the same bird. Where the differing areas adjoin, there may be mixture, but no true blending of colors or structural differences. Because of this independence chimeras may have certain advantages over the transplantation technique for the study of tissue relationships, since no tissue antagonism is to be expected.

GENERAL DISCUSSION

Comparison of flecking and chimerism

Flecking, like most cases of chimerism in pigeons, consists of irregular areas of recessive color in heterozygous birds which otherwise are of the dominant phenotype. The flecks rarely involve whole feathers but consist of patches on the feather, often of very small size. This would seem to indicate that the mutations occur late in ontogeny, indeed within the individual feather germs. The mutant areas in chimeras, on the other hand, must have their origin in mutations which have occurred much earlier, possibly in some cases even as early as the first cleavage of the egg (figures 10 and 11). If it occurred still earlier, namely in the germinal tissue of the parent, a mutant *individual* might result, and it is possible that Feldman's original "faded" came about in this way (see p. 18).

Mutations at the early stages which produce chimeras are relatively rare, even with genes, such as B^A, which show high inconstancy in the feather follicles. It must be supposed, therefore, that there is some internal condition at this late stage which predisposes to a high frequency of mutation in certain labile genes. Furthermore, the tendency would appear to increase with age, as indicated by the greater amount of flecking in old birds. The mutant areas in chimeras, on the other hand, have not been observed to change with successive molts,[3] which would indicate again that the spot is determined by a single earlier mutation.

The difference between flecking and the larger areas of the chimera is usually clear enough but the actual size of the spot in a chimera, as shown in E284E, figure 4, may be smaller than a large single-feather "fleck" (fig. 1). The difference is that the former covers an area of several feathers, so it is obvious the causative change ("mutation") must have occurred before differentiation of the individual feathers. Furthermore, even when it is small, the chimera spot appears to be constant through successive molts.

Mutation or physiological control

That internal physiological conditions may normally determine whether or not a gene will exhibit its characteristic phenotypic expression is definitely shown in an ordinary Barred Plymouth Rock feather, for example, where the action of the gene is alternately expressed and suppressed. The work of LILLIE and his associates has demonstrated that not only barring, but other patterns as well may be controlled with considerable accuracy by changing physiological conditions. It is not to be supposed in either of these cases that there has been corresponding genetic change in the cells concerned. Probably the same is true for all regular patterns. It has been

[3] One "reverse" exception, group 1, p. 25.

suggested that in the case of piebald patterns the white areas may be regions of somatic genetic change, but there seems to be no direct evidence in favor of such an explanation. Why then may not the flecking and chimerism described in this paper be explained as direct physiological response to local internal environment rather than to localized genetic change? Probably the strongest argument at the present time for the latter interpretation is the great irregularity of occurrence of the flecks and spots and the way they fit into a logical genetic scheme. Furthermore, the initial changes, particularly in the case of the larger chimera spots, must have occurred well back of the point where they are expressed, and they are not synchronized as the responses to direct physiological conditions so commonly are. The "half-sider" described and illustrated (figures 10 and 11) could scarcely be referred to a general physiological differentiation in the first two blastomeres, which was so permanent as to continue through all subsequent somatic cell generations. Such a result would, however, naturally follow from a genetic mutation in one of these blastomeres. From the half-sider to the bird with ordinary flecking there seems to be a series, representing mutations at different stages of development, which make it logical to apply the same interpretation to the latter type of marking. This does not preclude the possibility that changes in internal environment, such as that accompanying ageing, may have an influence on the frequency of somatic genetic change.

SUMMARY

Three sex-linked color factors in the pigeon, all probably allelic, and each dominant to wild type, are found accompanied in heterozygous males and the hemizygous females by a sort of variegation which we have termed flecking. This is usually most marked in males and in aged birds. Large flecks tend to be repeated in successive feathers from the follicles; small flecks less noticeably so. Flecks of recessive color have also been observed in connection with an autosomal factor, grizzle, but no special study of this condition has been made. The assumption of frequent recessive somatic mutation seems to account adequately for flecking.

Chimeras are treated in seven main groups based on the factors involved. The areas in chimeras which are discordant with the regular phenotype of the bird are commonly larger than the flecks, involving considerable patches of feathers. They may even include as much as half the bird, as in a "half-sider" described. Like the flecks, they mostly occur in heterozygotes, and in the majority of cases are recessive. These cases can also be explained as the result of recessive somatic mutations which have occurred relatively early in the ontogenetic development.

A few of the chimeras are not susceptible of this simple explanation.

These appear to involve dominant mutation, or possibly polyspermy. One multiple chimera described involves at least two color effects and two structural effects (counting arrangement of pigment as structural). It is suggested that at least one autosome was lost in this case.

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AN ANALYSIS OF X-RAY INDUCED CHROMOSOMAL ABERRATIONS IN TRADESCANTIA

KARL SAX

Arnold Arboretum, Harvard University, Cambridge, Massachusetts

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THE cause and nature of chromosomal alterations has become an important genetic problem since it has been shown that both mutation and speciation often are associated with structural alterations of the chromosomes (DOBZHANSKY 1937). The frequency of spontaneous structural changes is too low in most organisms to permit a statistical analysis of types or frequencies of alterations, although recently GILES (1940) has been able to analyze a considerable number of natural chromosomal aberrations in Tradescantia hybrids. In most respects these natural aberrations resemble those induced by X-rays, and although the spontaneous breaks are not caused by radiation, the same secondary factors seem to be involved in producing the various types and frequencies of aberrations. The X-ray induced aberrations can be produced in large numbers under controlled conditions, permitting adequate statistical analyses of types of aberrations and their frequency in relation to dosage, temperature, time of exposure, and radiation intensity.

The microspores of diploid species of Tradescantia provide excellent material for an analysis of X-ray induced chromosomal aberrations. The six chromosomes are large and the nuclear cycle can be timed rather accurately. During the summer months the microspore nucleus remains in the resting stage for about five days after the microspore is formed following meiosis. At about 30 hours before metaphase the chromosomes begin splitting, and at 23 to 24 hours before metaphase all chromosomes are split to form the sister chromatids. Thus the nuclear cycle from microspore formation to metaphase requires about six days. During the winter months the nuclear cycle is nearly twice as long and the prophase stage may begin at about 60–70 hours before metaphase. For an analysis of chromatid breaks induced at prophase the cells were fixed 24 hours after raying. Microspores fixed five days after raying were irradiated in the resting stage. Preparations were made at 48 hours after raying during the winter months, and 30 hours after raying during the summer months, for the analysis of very early prophase stages. The analyses of dosage, the time factor, and temperature effects were based on experiments done during the winter months. The division figures obtained provide a true random sample of aberrations because there is no chance of elimination of chromosomes or chromosome fragments.

The microspore cells were smeared, fixed in alcohol-acetic for a few

minutes, and stained with aceto-carmine. Several hundred division figures usually were obtained from each flower bud, and preparations with less than about a hundred metaphase or anaphase figures were discarded. Since there are six chromosomes in each microspore each preparation permitted the anlysis of from 600 to 2000 chromosomes. The results to be presented are based on an analysis of more than 300,000 chromosomes.

The statistical treatment of the data presents a difficult problem. Most of the individual values given in the various tables are based on the average frequencies on three slides, and the total number of chromosomes is from 3,000 to 6,000. Neither the number of slides nor the number of chromosomes provides the correct value of n for a critical analysis of variation. Accordingly we have taken the deviation from the mean, in percent of the mean, for each series of observations involving three or more slides each, and have obtained the standard deviation and probable error from these figures. The value of n was taken as the number of series times $n-1$ slides examined for each series. The generalized probable error is found to be about 10 percent of the mean. Many of the experiments were repeated several times with comparable results.

TYPES OF CHROMOSOMAL ABERRATIONS

Two general types of chromosomal aberrations are induced by X-rays, namely, chromatid breaks, and chromosome breaks. The chromatid breaks are induced at prophase when each chromosome consists of two sister chromatids, while the chromosome breaks are those induced during the resting stage when the chromosomes are in the form of single threads. Each of these general types of aberrations may be further classified into two groups: the aberrations caused by a single break which are referred to as one-hit breaks, and aberrations involving breaks in two different chromosomes or different loci of the same chromosomes which are referred to as two-hit aberrations. The origin and nature of these four classes of chromosomal aberrations is shown diagramatically in figure 1.

The one-hit chromatid breaks may involve one or both chromatids. The terminal deletion of one of the two chromatids (Plate I, figs. E and F) results in a chromatid fragment which is usually not included in either daughter nucleus at telophase. Often, however, the fragment appears to be separated from the centric arm only by an achromatic lesion, suggesting a partial breakage or imperfect reunion. Although these one-hit terminal deletions are rather frequent they cannot be scored accurately and have not been included in the analysis of X-ray breaks. The most frequent type of one-hit chromatid aberration is a break in both chromatids at the same locus followed by lateral fusion to produce a dicentric chromatid and an acentric U-shaped fragment (Plate I, B). At anaphase the dicentric chro-

matid forms a single bridge and the fragment tends to straighten out (Plate I, C). Such fragments are rarely included in the daughter nuclei. The ends of the fragment are the normal ends of sister chromatids (cf. figure 1).

The two-hit chromatid aberrations are of various types. The most frequent types are fusions between broken chromatids of two different chromosomes. These consist of reciprocal chromatid exchanges, producing a

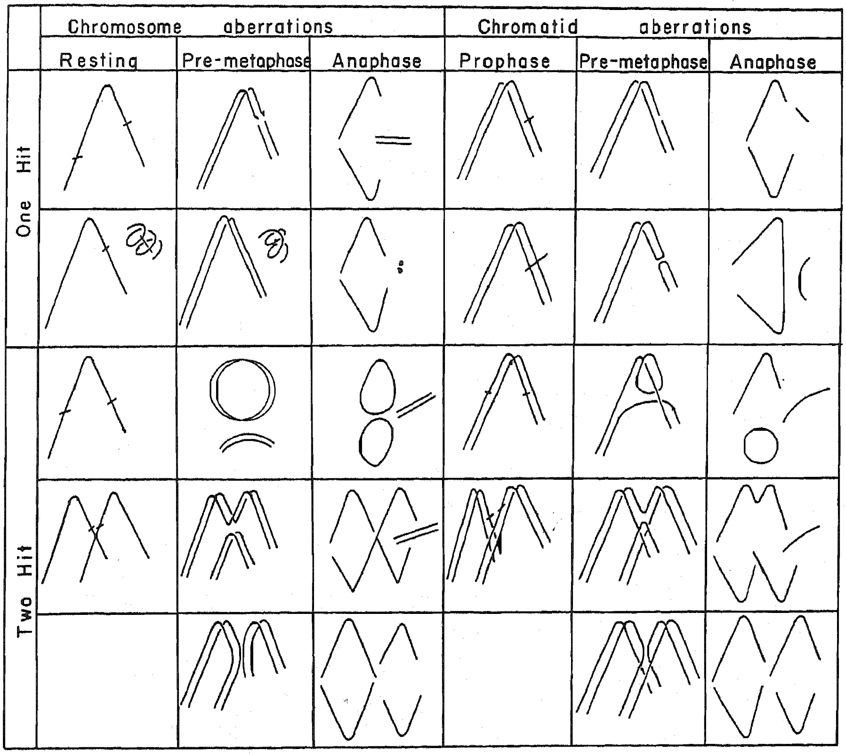

FIGURE 1.—The types of chromosomal aberrations induced by X-rays at prophase and at the resting stage. Only the types which can be recognized at metaphase or anaphase have been included, since they are the types used in the various analyses.

pseudo-crossover type of configuration (Plate I, D), or the broken ends may fuse to form a chromatid bridge between two chromosomes accompanied by an acentric fragment (Plate I, E). Other types have been found but they are rare. Occasionally the corresponding arms of a single chromosome are of unequal length at anaphase (Plate I, F) indicating an intercalary duplication such as KAUFMANN and BATE (1938) have described in Drosophila. Fusions between breaks in each of the two arms of a single chromosome have produced a few chromatid rings, and in rare cases an acentric ring is produced from two breaks in a single arm.

The chromosome aberrations include both one-hit and two-hit types. The one-hit aberrations include both intercalary and terminal deficiencies. The loss of a terminal segment of a chromosome results in a shortened arm with no fusion of the ends of sister chromatids, and the acentric fragment consists of two separate chromatids at metaphase (Plate I, G). The small intercalary deletions presumably are deficiencies produced by breaks in adjacent relic spirals during the resting stage (figure 1). These deleted fragments are small (Plate I, G and N) and have not been included in the analysis of chromosome breaks. Most of these deficiencies have been found to be two-hit aberrations (Rick, unpublished observations).

The two-hit chromosome aberrations include reciprocal interchange or fusion between different chromosomes and fusion or exchange between the arms of the same chromosome. Occasionally a relatively large intercalary deletion is produced resulting in an acentric ring fragment. Reciprocal interchange between arms of different chromosomes can be detected only when the exchange is unequal (Plate I, J), and such exchanges are infrequent. Fusions between broken ends of two chromosomes result in a dicentric chromosome and an acentric fragment (Plate I, H and I). The

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Description of Plate I

Photographs of Tradescantia microspore chromosomes showing the types of aberrations induced by X-rays. Compare with diagrams in figure 1.

Figure A.—Untreated,—six normal chromosomes at metaphase.

Figure B.—A one-hit chromatid aberration induced at prophase. Lateral fusions have occurred between the ends of the broken sister chromatids to produce a shortened arm with terminal fusion of sister chromatids and a U-shaped acentric fragment. Metaphase.

Figure C.—The same at anaphase showing the chromatid bridge and partially straightened U-shaped fragment.

Figure D.—A two-hit chromatid aberration. Reciprocal interchange between chromatids of different chromosomes.

Figure E.—The same, but with chromatids fused to form a chromatid bridge between chromosomes. Also a one-hit chromatid aberration—terminal deletion of only one of the two sister chromatids.

Figure F.—An intercalary chromatid duplication and deficiency involving the sister chromatids of one arm. Results in unequal arms at anaphase. Also a single chromatid terminal deletion.

Figure G.—Two one-hit chromosome aberrations. One is a terminal deletion of most of one arm, and the other a very small interstitial deletion.

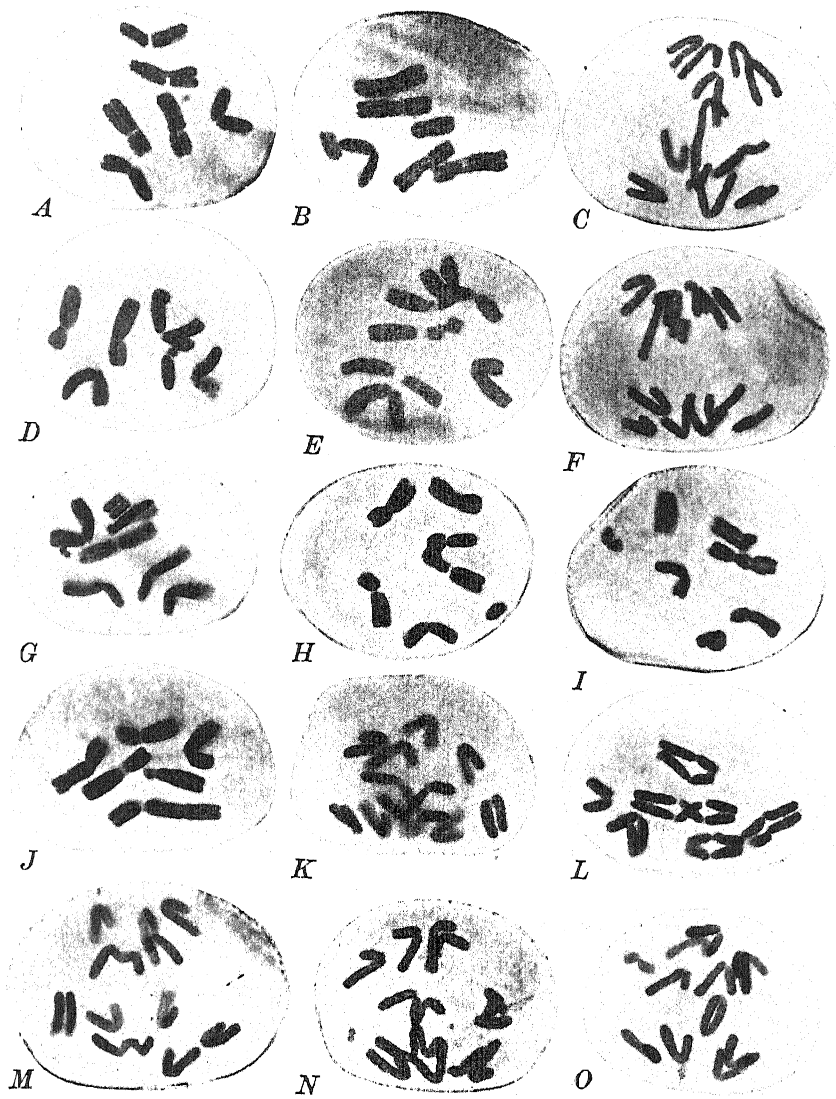
Figure H.—A two-hit chromosome aberration. A dicentric chromosome and the accompanying fragment. Note relational coiling between centromeres.

Figure I.—Two two-hit chromosome aberrations. A dicentric and a locked ring with their accompanying fragments.

Figure J.—Unequal interchange between two chromosomes.

Figures K–N.—Behavior of dicentric chromosomes at anaphase. Also a terminal chromosome deletion and an interstitial deletion in figure 14.

Figure O.—A continuous ring chromosome at anaphase. Also somatic non-disjunction of two anaphase chromosomes.



broken ends of the terminal deletions fuse to produce a fragment consisting of parts of two chromosomes. The ends of the fragment are the normal ends of the two arms. The relational coiling between centromeres of the dicentric chromosome persists to metaphase and at anaphase the dicentric chromatids may separate freely, form an X-shaped bridge, or interlock (Plate I, K–N). Fusion between breaks in the two arms of the same chromosome results in a ring chromosome and an acentric fragment (Plate I, I). The ends of the fragment are the normal ends of the two arms. These ring chromosomes may separate freely at anaphase, they may be locked, or they may form a single large ring (Plate I, O). Occasionally a ring chromosome is locked around its rod fragment.

Combinations of chromosome and chromatid breaks may occur in the same cell or even in the same chromosome, but they are very rare. The individual chromosomes must split very rapidly at prophase (SAX and MATHER 1939).

Other types of chromatid and chromosome aberrations undoubtedly occur, but inversions, transpositions or intra-chromosomal translocations, and simple intercalary translocations, either cannot be detected, or cannot be distinguished from reciprocal translocations. The types of aberrations included in the subsequent analyses consist of the one-hit chromatid breaks involving both chromatids, two-hit chromatid breaks which produce exchange or dicentric chromatids, and the two-hit chromosome breaks resulting in dicentric and ring chromosomes. These constitute most of the visible chromosomal alterations induced by X-ray doses used in the following experiments.

FREQUENCIES OF TYPES OF ABERRATIONS IN RELATION TO SPATIAL ARRANGEMENT OF THE CHROMOSOMES

The relative frequencies of one-hit and two-hit aberrations vary with the X-ray dosage and will be considered later. The various types of two-hit aberrations, both chromosome and chromatid, appear in the same relative frequencies at different dosages, even though the actual frequencies increase exponentially with dosage. The relative frequencies of the two-hit aberrations provide evidence regarding the conditions and limitations of fusion between broken ends of chromatids and chromosomes. The frequencies of types of chromatid and chromosome alterations are shown in table 1. The dicentric chromatids are more than twice as frequent as the exchange chromatids. The exchange types cannot ordinarily be recognized at anaphase but most of these observations were made at metaphase so that the 2:1 ratio is approximately correct. The ratio of dicentric and ring chromatids is about 13 to 1, but for the chromosome aberrations the ratio of dicentrics and rings is 3.3 to 1.

The loci of breaks

Usually the exchange breaks are at similar loci in respect to the centromeres of the two chromosomes. An analysis of a random sample of dicentric and exchange chromatid aberrations showed 33 with breaks in both chromatids at about the same relative loci and only six which involved breaks at clearly different loci in the two chromosomes. A similar condition must obtain for chromosome breaks because unequal translocations are seldom observed.

The distribution of X-ray induced breaks must be at random among the chromosomes and at random for the loci of any one chromosome. But the

TABLE 1

Frequencies of types of chromatid aberrations; 24 hours, 40–200 r.

TOTAL CHROMOSOMES	ONE HIT		TWO-HIT BREAKS					
	DELETION	%	EXCHANGE	%	DICENTRIC	%	RING	%
9906	468	4.7	182	1.8	402	4.1	30	0.3

Frequencies of types of chromosome aberrations; 5 days, 100–500 r.

TWO-HIT ABERRATIONS			
TOTAL	DICENTRICS	RINGS	RATIO
10,344	7,936	2,408	3.3:1

aberrations resulting from these breaks are not at random for the various loci of the chromosome arms. The frequency of both chromosome and chromatid breaks which result in aberrations is greatest at the proximal end of the chromosome arm. The distribution of such chromatid and chromosome breaks is shown in graphic form in figure 2. The data were derived from an analysis of 340 chromosome aberrations and 334 chromatid aberrations (SAX and MATHER 1939). The loci of breaks were divided into five groups; group one including those in the proximal fifth of the chromosome arm and the subsequent groups including the more distal loci. The difference in distribution of chromatid and chromosome breaks is statistically significant according to the χ^2 test, but technical difficulties in determining breakage points in dicentric chromosomes may account for this difference.

THE RELATION BETWEEN X-RAY DOSAGE AND FREQUENCY OF CHROMOSOMAL ALTERATIONS

The relation between the X-ray dosage and mutation rate in Drosophila has been shown to be linear by a number of investigators (TIMOFÉEFF-

RESSOVSKY 1937). Recent work on both Drosophila and Tradescantia shows that the relation between X-ray dose and chromosomal aberrations is not linear. OLIVER (1932), using genetic methods, was the first to show an exponential increase in chromosomal alterations with increased dosage in Drosophila. Direct cytological analysis of the salivary gland chromosomes of Drosophila also shows an exponential increase in chromosome changes with dosage (BAUER, DEMEREC and KAUFMANN 1938). MULLER (1938) also refers to experiments which indicate that the frequency of both translocations and inversions varies as the 3/2 power of the X-ray dose. The frequency of chromosome aberrations in Tradescantia plotted against dosage also shows an exponential curve (SAX 1938).

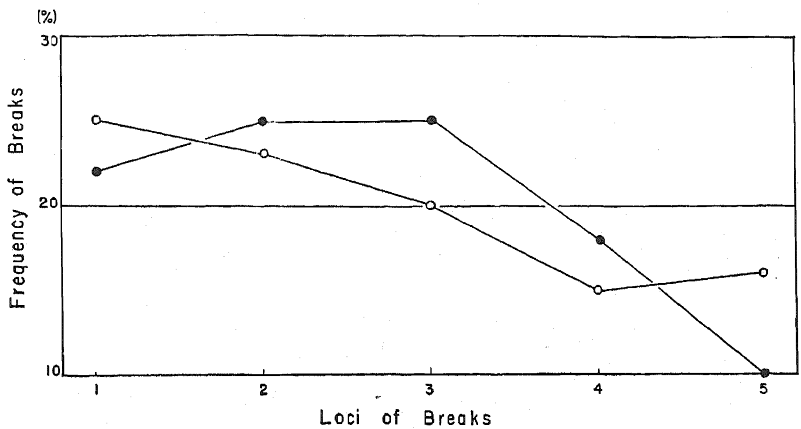


FIGURE 2.—Loci of chromosome and chromatid breaks involved in X-ray induced aberrations. Locus 1 includes the first fifth of the chromosome arm next to the centromere and locus 5 the distal fifth. If such breaks were at random 20 percent of all breaks should be found at each of the 5 loci. Data from SAX and MATHER (1939). ○ = chromatid. ● = chromosome.

In all these experiments the frequency of chromosome aberrations increased approximately as the 3/2 power of the dosage. If each aberration is dependent upon two breaks, as is probably true in all these experiments, the frequency of aberrations should increase as the square of the dosage. In all these previous experiments the dosage was increased by increasing the time of exposure. Recently it has been found that the frequency of two-hit chromosomal aberrations is dependent upon the time-intensity factor, and that a high intensity of dosage is more effective in producing aberrations than the same dose given slowly (SAX 1939). Accordingly we should vary the dosage by varying the intensity rather than by varying the time of exposure.

In the following experiments on the relation between X-ray dosage and frequency of chromosomal aberrations, the dosage was varied by changing

the distance between the target and the flower buds according to the inverse square law, so that variation in dosage was given without changing the exposure time. The minimum distance used was 24 cm, but even at this distance there is some variation in dosage, due to slight differences in the positions of the young buds in the inflorescences. At greater distances this experimental error is decreased. The source of the X-rays was a Coolidge tube with a tungsten target, operated at 10 ma, 134 K.V. with no screen.

The results of increased dosage on frequency of chromosomal aberrations induced at the resting stage are shown in table 2. The frequency of these

TABLE 2

The relation between X-ray dosage and chromosome aberrations; time constant.

DOSE IN R UNITS	TOTAL CHROMOSOMES	CHROMOSOME ABERRATIONS*				
		DICENTRIC	RING	TOTAL	%B	$(D/107)^2$
100	1800	5	1	6	0.7	0.8
200	1710	21	8	29	3.4	3.5
300	1464	48	13	61	8.3	7.8
400	1902	104	28	132	13.9	14.0
500	1530	134	25	159	20.8	21.8

* Each aberration involves two breaks.

two-hit aberrations increases as the square of the dosage. The equation for the curve is percent breaks, B, equals $(D/107)^{2.0}$, where D is the dosage in r units and 107 is a constant. The values of the exponent and constant were derived by plotting the logarithm of the dosage against the logarithm of the percent of effective breaks and obtaining the values from the graphs.

The low frequency of one-hit chromosome breaks induced at the resting stage does not permit an adequate comparison of the effects of varying dosage on one-hit and two-hit chromosome aberrations, so for this comparison we have used one-hit and two-hit chromatid aberrations induced at prophase. The frequency of chromatid breaks is much higher than that of chromosome breaks and it was not possible to analyze accurately the chromatid breaks induced at a dosage of much over 200 r. On the other hand it was possible to obtain an appreciable number of chromatid aberrations at 10 r. The effect of varying dosage on both one-hit and two-hit chromatid aberrations is shown in table 3. The frequency of the two-hit chromatid aberrations increases approximately as the square of the dosage. The equation for the curve is percent $B=(D/67)^{1.9}$. The one-hit aberrations, however, show an approximately linear relation to dosage, the equation being percent $B=(D/45)^{1.1}$. The deviations from the expected value

of the exponents may be due to experimental error since the data in this table were derived from three different series of experiments extending over a period of several months. It is possible that environmental conditions or the output of the X-ray tube may have varied sufficiently so that

TABLE 3

The relation between X-ray dosage and one-hit and two-hit chromatid breaks; time constant.

DOSE IN R UNITS	TOTAL CHROMOSOMES	CHROMATID ABERRATION BREAKS					
		1-HIT	%B	$(D/45)^{1.1}$	2-HIT	%B	$(D/67)^{1.9}$
10	6330	14	0.2	0.2	2	0.03	0.02
20	6930	24	0.4	0.5	4	0.06	0.08
40	8610	76	0.9	1.1	34	0.4	0.31
80	7368	148	2.0	1.9	112	1.5	1.4
120	4902	141	2.9	2.9	124	2.5	3.0
160	8292	375	4.4	4.0	422	5.1	5.3
200	8508	472	5.1	5.1	685	8.1	8.1

the data from different experiments were not strictly comparable. It is also possible that some of the one-hit types of aberrations were produced by two adjacent independent hits. In general, however, it is apparent that "one-hit" aberrations show a linear response to dosage, while the "two-hit" aberrations vary as the square of the dosage, when the exposure time is held constant.

The effect of increased dosage on the frequencies of the various types of aberrations is shown in the form of curves in figure 3. The curves for the chromatid aberrations have been extrapolated beyond 200 r for comparison with the two-hit chromosome aberration curve. The frequency of the two-hit chromatid breaks is considerably greater than that of the two-hit chromosome breaks. The two frequencies are not entirely comparable, however, because the reciprocal interchanges can be detected when induced at prophase, but only rarely when induced at the resting stage.

The relative frequencies of one-hit and two-hit chromosomal aberrations vary not only with dosage, but also with the time of prophase development at the time of irradiation. At very early prophase the exchange or fusions between chromatids of different chromosomes are much less frequent in relation to the number of terminal deletions than at later prophase stages. The relative frequencies of these one-hit and two-hit chromatid aberrations and the two-hit chromosome aberrations induced at early prophase, 48 hours before metaphase, are shown in table 4. The theoretical frequency of two-hit chromatid breaks is based on the relative frequencies of one-hit and two-hit aberrations produced at mid-prophase, while the theoretical frequency of the chromosomal aberrations is derived from the equation

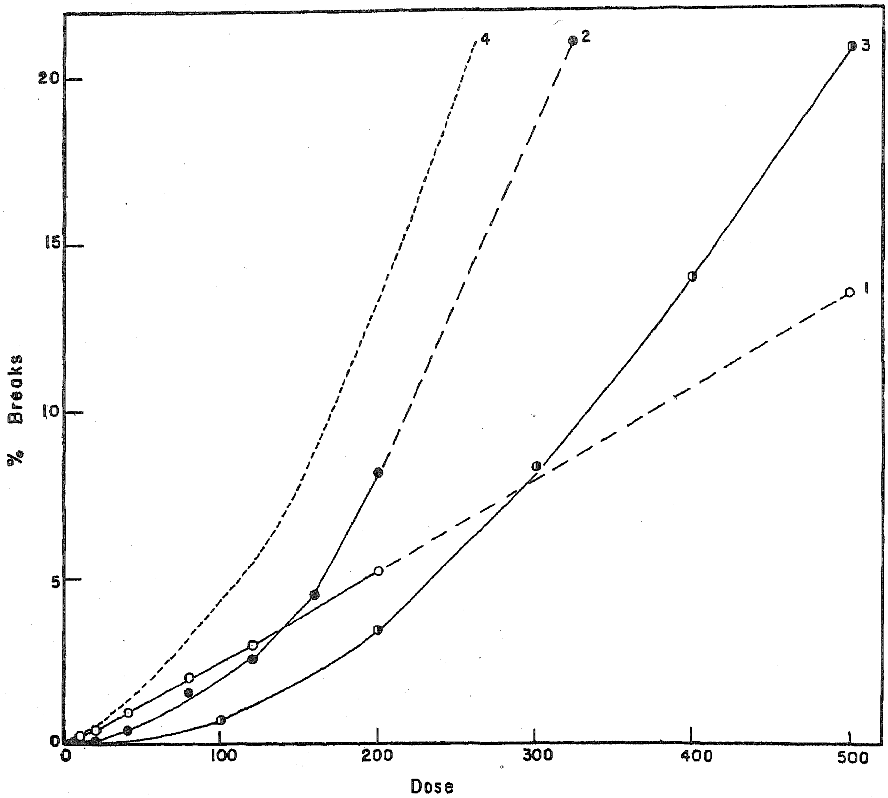


FIGURE 3.—The relation between the frequencies of chromosome and chromatid aberrations and the X-ray dosage in r units. Time of exposure constant. The curves for the one-hit and two-hit chromatid breaks have been extrapolated beyond 200 r. The equations for the curves are: (1) one-hit chromatid breaks, percent $B=(D/45)^{1.1}$, (2) two-hit chromatid breaks, percent $B=(D/67)^{1.9}$, (3) two-hit chromosome breaks, percent $B=(D/107)^{2.0}$ and (4) total chromatid breaks, percent $B=(D/37)^{1.5}$.

percent $B=(D/107)^2$, assuming that all chromosomes are single. The data show that most of the chromosomes were not yet split at this time. The aberrations induced in the split chromosomes show a much lower frequency of two-hit aberrations than would be expected at mid-prophase.

TABLE 4

Frequencies of one- and two-hit chromatid breaks induced at very early prophase; 48 hours.

DOSE	TOTAL CHROMOSOMES	CHROMATID					CHROMOSOME		
		ONE-HIT	%	TWO-HIT	%	THEOR.	TWO-HIT	%	THEOR.
160 r	3276	26	0.8	8	0.2	1.0	90	2.7	2.3
320 r	3030	46	1.5	6	0.2	3.8	252	8.3	9.0

The time-intensity factor

An analysis of the time-intensity factor in the X-ray production of various types of chromosomal aberrations has shown that the two hits necessary to produce an alteration involving two chromosomes need not occur simultaneously, but may occur over a period of time as long as an hour (SAX 1939). The time-intensity factor was varied by giving equal doses at different intensities, or by giving the same intensity of radiation over various periods of time by intermittent exposures. The intensity of radiation was varied by placing the inflorescences at various distances from the target according to the inverse square law. The results of the time factor at different X-ray intensities are shown in graphic form (figure 4). The

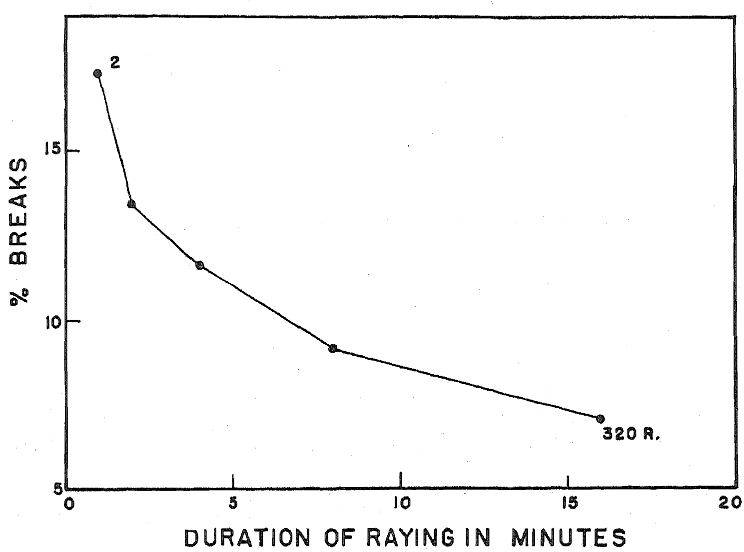


FIGURE 4.—The relation between the frequency of chromosomal aberrations and the intensity of X-radiation. The intensity of radiation was varied from 320 r/m to 20 r/m.

dosage was 320 r and the intensity varied from 320 r/m to 20 r/m according to the time of exposure. The curve is based on analysis of 10,620 chromosomes. The chromosomes which were given 320 r in one minute show twice as many aberrations as those which were given 320 r in sixteen minutes. Similar experiments using other total dosages showed similar results.

Since the chromosome aberrations used in this analysis include only two-hit alterations, the greater frequency of such aberrations at high X-ray intensity must mean that reunion of broken ends occurs within certain limits of time. If the broken ends fuse quickly a low intensity should produce fewer aberrations than a high intensity of radiation be-

cause a break in one chromosome would heal before a second break occurred in an adjacent chromosome, and no aberration would be produced. But if the broken ends remain in an unstable condition for a long time and

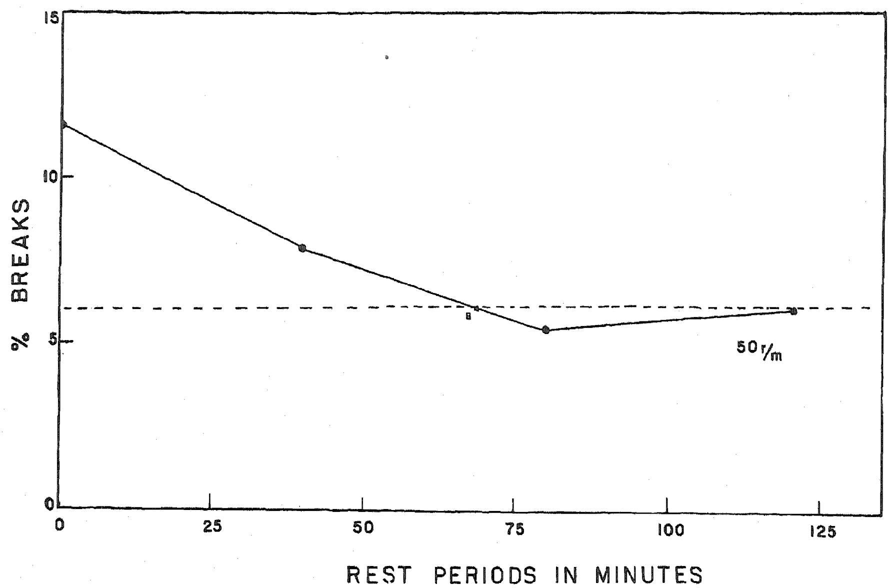


FIGURE 5.—The relation between frequency of chromosomal aberrations and intermittent radiation. Four two-minute X-ray doses were given at 50 r/m, with rest periods of 0, 40, 80 and 120 minutes respectively. The base line (B), established by determining the percentage of breaks for a single two-minute exposure and multiplying by 4, coincides with percent of breaks at about the 70 minute rest period.

few fusions occur during the time of raying there should be little effect of the time-intensity factor. The results show that many fusions occur in a few minutes and probably in even less time, but they do not indicate the maximum length of time necessary to complete the fusion of all broken ends.

The time during which a broken end of a chromosome remains in an unstable condition can be determined from intermittent timing experiments. The results of one of these experiments are shown in the form of a curve in figure 5. Five groups of flower buds were irradiated, all at 50 r/m. The first group was given continuous radiation for eight minutes, the second, third, and fourth groups were given four intermittent exposures of two minutes each, with rest periods of 40, 80, and 120 minutes respectively. The fifth group was given only one two-minute exposure. If broken ends of chromosomes can remain unstable and capable of fusion with other broken ends for only a short time, a sufficiently long rest period should prevent fusions between breaks induced at successive exposures. This point

is reached where a further increase in the rest period produces no further decline in frequency of aberrations. The base line can also be determined by multiplying the frequency of aberrations induced by a single exposure by the number of exposures used in the intermittent timing experiment. In the above experiment the base line is at about six percent and is reached at a rest period of somewhat more than an hour. These results show that a break induced at one exposure period may remain open so that a broken end can fuse with another broken end produced by the second exposure an hour later, although most of the fusions occur in a considerably shorter period. Similar experiments with different X-ray intensities show similar results, although at high intensities, where the dose is relatively high for each exposure, there is less effect of the intermittent exposures. These results are based on an analysis of more than 80,000 chromosomes.

The effect of the time-intensity factor is based on the fact that the two-hit aberrations are dependent upon two independent breaks, limited in time of occurrence. According to this interpretation the time-intensity factor should have no effect on one-hit aberrations. This assumption has been tested by comparing the effect of intermittent timing on one-hit and two-hit chromatid aberrations. As expected, the frequency of the one-hit aberrations is independent of the time factor while the frequency of the two-hit aberrations declines with increasing rest periods (SAX 1939).

THE EFFECT OF TEMPERATURE ON X-RAY INDUCED CHROMOSOMAL ABERRATIONS

In an earlier experiment no differences in frequency of chromosomal aberrations were found when the cells were irradiated at different temperatures (SAX 1938). The resu ts were not based on an analysis of individual chromosomes and the frequency of aberrations included all types of alterations obtained at various times after irradiation. More critical experiments show clearly that the temperature during and following irradiation has a marked effect of the frequency of both one-hit and two-hit aberrations (SAX and ENZMANN 1939). The results of two of these experiments are shown in graphic form in figure 6.

The inflorescences were immersed in water at the desired temperature for five to ten minutes before raying and kept just under the surface of the water during irradiation. In a few experiments the flower stalks were removed from the water and placed at room temperature soon after raying, but usually they were left in the water for an hour after raying. When pasteboard cartons were used for containers the temperature dropped gradually during this period, but similar temperature effects were obtained when the flowers were put in thermos bottles where the temperatures were maintained at a constant level during the entire period.

The cells which were held at a low temperature during and following irradiation had from two to five times as many chromosome aberrations as those which were subjected to a high temperature. Preliminary work on the frequency of aberrations at various temperatures shows that the frequency does not decrease regularly with increased temperature, but tends to drop sharply at a critical temperature, which varies from less than 20°C to above 30°C, depending on the time of year the work is done. Presumably this seasonal effect is related to the length of the nuclear cycle; in the winter months the nuclear cycle from meiosis to division of

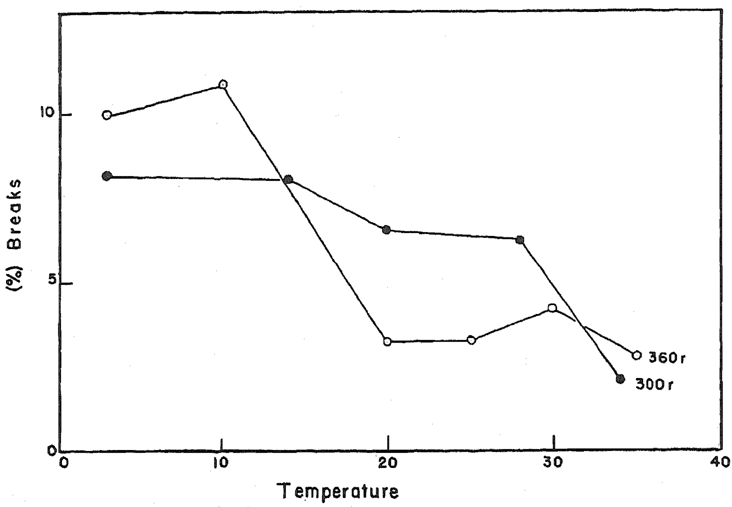


FIGURE 6.—The effect of varying temperatures during and immediately after X-radiation on the frequency of chromosomal aberrations. The different temperatures at which the greatest decrease in frequency of aberrations occur in the two curves is attributed to seasonal effects (Data from SAX and ENZMANN 1939).

the microspore nucleus requires about 12 days and the critical temperature is about 30°, while in late spring the nuclear cycle requires about one week and at this season the critical temperature is less than 20°.

At both mid-prophase and at the resting stage the frequency of X-ray induced aberrations is decreased at high temperatures, but at earliest prophase when the chromosomes are beginning to split into chromatids the higher temperatures cause many more aberrations than are produced by raying at low temperatures. The effect of high (30°C) and low (3°C) temperatures on frequency of X-ray induced aberrations at different prophase stages is shown in table 5. The low frequency of chromatid aberrations induced at early prophase, 48 hours before metaphase, is due to the fact that many of the chromosomes have not split and these produce chromosome aberrations which are not included in this table. The frequency of X-ray induced aberrations, at 24 hours before metaphase, is much higher

in the cold series, but at 48 hours the high temperature produces many more aberrations than the low temperature. This relation holds for both one-hit and two-hit aberrations, although the latter are infrequent in the 48 hour preparations.

INTERPRETATION OF RESULTS

The time of chromosome splitting

The evidence regarding the time of chromosome splitting in the nuclear cycle has been reviewed by MATHER (1937). The results obtained from X-ray experiments are considered to be more critical than the interpretations based on direct visual evidence. Certainly the types of aberrations induced by X-rays at the various stages in the nuclear cycle of Tradescantia microspore indicate that the chromosome reacts as a single thread

TABLE 5

The effect of temperature on frequency of chromatid aberrations at very early prophase (48 hours) and at midprophase (24 hours). Total chromosomes 19,848.

		CHROMATID ABERRATIONS			
		% ONE-HIT	% TWO-HIT	TOTAL	DOSAGE
24 hours	Cold	3.6	2.6	6.2	160 r
	Hot	1.8	1.6	3.4	
48 hours	Cold	0.8	0.1	0.9	
	Hot	1.1	0.4	1.5	
48 hours	Cold	1.5	0.2	1.7	320 r
	Hot	4.6	1.0	5.6	

during the resting stage and becomes effectively split into sister chromatids at prophase (cf. RILEY 1936). It may be argued that the effect of a single X-ray quantum is not sufficiently localized to be used as a tool for differentiating the number of chromatids or half-chromatids in a chromosome, since a single "hit" can break two adjacent chromatids at late prophase when the sister chromatids are more than a tenth of a micron apart. If the chromosomes were double at the early resting stage a single hit would usually break both of the closely associated chromatids, but such breaks should be followed by lateral fusion of broken ends of adjacent sister chromatids. Aberrations of this type are very seldom found in microspores irradiated in the resting stage, and the rare exceptional cases can be attributed to spontaneous aberrations. The effective split which produces differentiated chromatids, corresponding to those involved in crossing over at meiosis, certainly occurs at the prophase stage in the Tradescantia microspores.

There is some experimental evidence that the effective split may occur

at the resting stage preceding nuclear division (MATHER 1937), or even at the preceding anaphase. The behavior of the broken ends of inversion bridge chromosomes suggests that the meiotic anaphase chromosomes may be effectively split. In both Tradescantia (SAX 1937) and Zea (McCLINTOCK 1938b) the break in an inversion bridge is followed by lateral fusion of sister chromatids to produce a bridge at the following division of the chromosome in the microspore. If the chromosome were single at meiosis a single broken end would have no other broken end with which to fuse, and perhaps such a broken end would remain in an unstable condition until the chromosome is split at the prophase stage of the microspore nucleus, at which time the lateral fusion would occur between the broken ends of sister chromatids. But McCLINTOCK has found that adjacent double inversion bridges behave in the same way, even though two broken ends are present in the newly formed microspore nucleus. On the other hand a break in a somatic ring chromosome of Zea is followed by fusion of broken ends of the two arms and not between ends of adjacent sister chromatids (McCLINTOCK 1938a). This difference cannot be attributed to differences in behavior at meiosis and mitosis because X-ray induced aberrations in Tradescantia root tips produce double bridges, which, when broken, are followed by lateral fusion of ends of sister chromatids. There is no reason to believe that the X-ray induced and spontaneous breaks differ in their effects on subsequent fusions of broken ends of chromosomes.

The behavior of the induced ring chromosomes is of interest in relation to the nature of splitting. If these ring chromosomes induced at the resting stage are single threads they must split in different planes because the rings may be continuous or interlocked at the following metaphase. This problem has been analyzed in some detail by HUSTED (1936).

The time of effective chromosome splitting in different microspores is quite variable, ranging from about 23 to 31 hours before metaphase in Tradescantia plants grown in the field during the summer months. The splitting of the chromosomes of a single nucleus is much more limited in time, and the splitting of an individual chromosome must be very rapid since both chromosome and chromatid breaks are rarely found in the same chromosome. There is some indication that effective splitting begins at the proximal end of the chromosome arm and progresses distally (SAX and MATHER 1939).

The frequency of types of aberrations in relation to chromosome arrangement

It has been shown that all potential fusions between broken ends of chromosomes are completed within an hour after the breaks are produced. There can be no extensive movement of the chromosomes during this

time, at most periods in the nuclear cycle, so that fusions between broken ends must be limited to those in close proximity. Certainly at the resting stage there could be no extensive movement of the chromosomes, which in the form of relic coils appear to completely fill the nucleus (BELAR 1928). With an increase in nuclear size and uncoiling of relic spirals at prophase more movement of the chromosomes could occur, but not sufficient to permit random association of broken ends of chromatids. Accordingly, an analysis of the loci of breaks involved in fusions between different chromosomes or chromosome arms should indicate the arrangement and relations of the chromosomes at various phases in the nuclear cycle.

The chromosomes of Tradescantia are strongly polarized during the resting and early prophase stages so that if little chromosome movement occurs between breakage and fusion, the fusions between different chromosome arms should occur usually at corresponding loci in respect to the distance from the centromeres. Such a relation is found for both chromosome and chromatid aberrations. Unequal exchanges between chromosome arms are rare, and an analysis of the fusions and exchanges between chromatids of different chromosomes shows that the breaks have occurred at approximately the same relative loci of both chromosomes in over 80 percent of the figures examined.

The relative frequencies of dicentric and ring chromosomes also show that proximity facilitates, or conditions, the fusions of broken ends of chromosomes. A broken end of a chromosome arm can fuse with a broken end in the other arm of the same chromosome to form a ring, or it can fuse with a break in any one of the ten arms of the other five chromosomes to form a dicentric union. Random reunion of broken ends should produce a ratio of dicentric to ring chromosomes of 10:1, but the observed ratio is 3.3 to 1 (table 1). It is evident that the distance between breaks, both laterally and longitudinally in respect to the polarized arms of the chromosomes, conditions or determines the occurrence of two-hit aberrations.

The spatial relationships of chromosome arms appear to change as the nuclear cycle progresses from the resting stage into prophase. At the resting stage the ratio of dicentric to rings is 3.3:1, but irradiation at prophase results in a ratio of dicentric to rings of 13:1. This decrease in frequency of ring chromosomes at mid-prophase is attributed to the greater separation of the two arms of a single chromosome at this stage. It is known that chromosomes repel each other, especially when they become split into sister chromatids. The prophase chromosomes also become shorter and the increased size of the nucleus permits greater movement of the chromosomes.

The relative frequencies of one-hit and two-hit aberrations induced at prophase vary with dosage as is shown in figure 3. There is, however, a

striking difference in these frequencies between early and mid-prophase (table 4). The relative frequency of two-hit aberrations induced at early prophase is very much less than at mid-prophase. This difference can hardly be attributed to greater repulsion between arms of chromosomes, and must be related to differences in the nature of the induced breaks. The two-hit breaks—dicentrics, exchanges and rings—induced at mid-prophase may depend upon breaks in only one chromatid of each of the chromosome arms involved in the aberrations. The sister chromatids at this stage are distinctly separated and breaks in only one of the two sister chromatids at a given locus are found at metaphase. At earliest prophase, however, the two sister chromatids must be closely associated so that a single X-ray hit would usually break both chromatids at the same locus. Such breaks would be followed by the fusion of adjacent ends of the broken chromatids in most cases, to produce a terminal deletion which is classed as a one-hit aberration. This interpretation is supported by the fact that two-hit chromosome aberrations, induced before chromosome splitting, but at about the same time, show a normal frequency.

The limitations of fusions imposed by the factor of proximity must mean that most of the breaks induced by X-rays do not lead to the production of aberrations. The two broken ends simply reunite in the original position and no structural alteration of the chromosome is visible at later stages in nuclear development. The chromosome breaks which lead to fusions between chromosome arms must constitute only a very small proportion of the total breaks induced, but simple breaks at the following metaphase are far less frequent than aberrations which involve two breaks in close proximity. The one-hit breaks at prophase are frequent only because two chromatids are broken and lateral fusion of broken ends leads to a permanent aberration. The frequency of breaks which heal in the original position cannot be determined, but such breaks must be far more frequent than those which lead to gross structural changes in the chromosomes.

A comparison of the frequencies of types of aberrations has also been used in the analysis of chromosome arrangements in Drosophila sperm (CATCHESIDE 1938; BAUER, DEMEREC and KAUFMANN 1938). The salivary gland chromosomes permit an analysis of alterations within chromosome arms, as well as between chromosome arms, although the numerous cell divisions between irradiation and chromosome analysis must produce differential elimination of the various types of aberrations. CATCHESIDE finds that exchanges within the same arm are twice as favored as those between different chromosome arms. Exchanges between arms of the same chromosome when compared with the frequency of exchanges between arms of different chromosomes, show no significant deviation from a random dis-

tribution. Similar results have been obtained by BAUER, DEMEREC, and KAUFMANN. These results must mean that a single chromosome in the Drosophila sperm is folded so that various parts of the same chromosome arm are in close proximity. Presumably, little movement is possible in the closely packed chromosomes of the sperm and induced aberrations must depend upon close proximity of the induced breaks.

The loci of breaks in relation to aberrations

It has been shown that the distribution of breaks involved in chromosome and chromatid aberrations is not at random for the various loci of chromosome arms. The initial breaks must be at random, but those which are involved in chromosome alterations are most frequent at the proximal ends of the chromosome arms. This distribution must be caused by secondary factors, and since the unequal distribution holds for one-hit chromatid breaks the secondary factor must be confined to the individual arms of the chromosomes. The greater frequency of such breaks at the proximal end of the chromosome arm is attributed to stresses imposed by the various coiling mechanisms which would tend to throw the broken ends out of alignment so that reunion in the original position would be inhibited. The breaks induced in the chromosomes of the sperm of Drosophila show a nearly random distribution of breaks with a possible higher frequency at the distal ends (BAUER, DEMEREC and KAUFMANN 1938). Perhaps the more random distribution of effective breaks in Drosophila can be attributed to the close association of the chromosomes in the Drosophila sperm so that proximity of broken ends does not necessitate any appreciable movement of broken ends in order to produce fusions between different chromosomes.

The relation between X-ray dosage and frequency of chromosomal aberrations

In both Drosophila and Tradescantia the frequency of X-ray induced breaks increases approximately as the 1.5 power of the dosage when the various doses are given at the same intensity. But since the time factor must be considered in such experiments the dosage should be varied by changing the intensity and not the time of exposure. When this is done the frequency of two-hit chromosome and chromatid aberrations in Tradescantia varies approximately as the square of the dosage. This relation should be expected since the chance that two or more independent events will happen together is the product of their respective chances of happening.

The frequency of simple deletions is assumed to be induced by single hits and should show a linear relation to dosage, at least for doses which

produce a low frequency of breaks. The data indicate a slight exponential increase of aberrations with increased dosage which may be due to experimental error, or to the occasional production of the deletions by two independent hits in the two chromatids at approximately the same locus.

The number of X-ray hits or quanta necessary to produce an effect has been calculated by comparing the survival curves with those obtained from Poisson's exponential series (TIMOFÉEFF-RESSOVSKY 1937). For one-hit and two-hit chromosomal aberrations the equation can be expressed as: percent $B = 1 - e^{-x}$ and percent $B = 1 - e^{-x}(1 + x)$, respectively, where x represents the number of effective hits. Using an arbitrary value of x of .00025 per r unit of dosage, we find that the calculated values for the one-hit aberrations are practically identical with the observed values. The calculated values of the two-hit curve also are in close agreement with the observed percentages of breaks at the various doses, but in order to get these theoretical values, the value of x must be increased to .0025 per r unit. The dosage curves, $(D/45)^{1.1}$ and $(D/67)^{1.9}$, do not indicate such different dosages for one- and two-hit aberration frequencies, as is indicated by the Poisson exponential series. It is not clear how these apparent discrepancies can be reconciled. It is evident, however, that the slopes of the two dosage curves are practically identical with the theoretical curves for one-hit and two-hit effects.

In all of the various experiments on the relation between dosage and the mutation rate in Drosophila a linear relation has been found. The frequency of mutation is also independent of the time-intensity factor (TIMOFÉEFF-RESSOVSKY 1937). These facts indicate that most of the induced mutations are produced by single hits and that relatively few of the induced mutations are due to "position effects" resulting from two-hit aberrations such as reciprocal translocations and large inversions.

The frequency of X-ray induced aberrations induced in the Drosophila sperm must also depend on the time factor. With dosage controlled by increasing the time of exposure the relation between frequency of aberrations and dosage is expressed by the equation percent $B = (D/K)^{1.5}$ in both Drosophila and Tradescantia; but in Tradescantia, with the time of exposure constant, the frequency of aberrations varies as the square of the dosage. Control of the time factor should also increase the exponent from 1.5 to 2.0 for the dosage-aberration curve in Drosophila.

The differential susceptibility of chromosomes to X-rays during the nuclear cycle and in different types of cells

The frequency of gross chromosomal aberrations is much greater at prophase than during the resting stage, when the same X-ray dose is used (figure 3). The equation for the relation between dosage and frequency of

aberrations is: percent $B=(D/37)^{1.5}$ for all the chromatid aberrations recorded, and percent $B=(D/107)^2$ for the chromosome aberrations. This comparison may not be entirely valid because certain types of aberrations can be detected when induced at prophase, but not when induced at the resting stage. In general, however, the frequency of gross alterations is greatest when the cells are rayed at prophase. This relation is to be expected for the following reasons: there are twice as many threads to be hit at prophase; terminal deletions can be produced at prophase by lateral fusion between broken chromatids while in the resting stage they are produced only when the broken end loses its capacity for fusion; and the secondary factors of chromosome torsion and movement would be expected to be greater at prophase.

A comparison of the frequency of chromosomal aberrations in Tradescantia and Drosophila shows that breaks involving chromosome changes are much more frequent in Tradescantia microspores than in Drosophila sperm. Some of this difference may be attributed to inviable aberrations in Drosophila, since the chromosomes were analyzed many cell generations after raying the sperm. The data of BAUER, DEMEREC and KAUFMANN show that the frequency of breaks, in percent of total chromosomes, may be expressed by the equation, percent breaks $=(D/1150)^{1.5}$. For Tradescantia microspores rayed at the resting stage, with dosage varied by varying the time of exposure, the equation is: percent $B=(D/80)^{1.5}$ (SAX 1938). These data would seem to indicate that the Tradescantia chromosomes are much more sensitive than those of Drosophila. This difference probably can be attributed to differences in chromosome size in the two genera and to differences in chromosome organization in the two types of cells. According to METZ and BOCHE (1939) both chromosome aberrations and mutations in Sciara are less frequent than in Drosophila when given the same X-ray dosage.

The difference in the frequencies of X-ray induced chromosomal aberrations in different cells of the same organism should provide additional evidence regarding X-ray effects on the chromosome. It is known that both mutations and gross chromosomal alterations occur more frequently in the sperm of Drosophila than in the oocytes for a given X-ray dose (OLIVER 1934). In Sciara gross chromosomal alterations are readily secured by raying the sperm, but were not found when the egg cells were irradiated. METZ and BOCHE (1939) attribute this difference in Sciara to physical differences in the chromosomes of the two types of cells. The close proximity of the chromosomes in the sperm should facilitate illegitimate union of broken ends, while most of the chromosomes of the eggs at the time of treatment were in metaphase or anaphase of the first meiotic division. The diffuse stage at meiotic prophase of meiosis also was found to

be resistant to X-ray effects. METZ (1934) has suggested that the chromosome matrix plays an important part in this differential susceptibility of chromosomes to radiation.

The differential susceptibility to X-rays of different stages of the nuclear cycle, of different cells in the same organism, and of similar cells in different organisms may depend in part on the same secondary factors such as proximity of chromosomes, freedom of chromosome movement, and the physical condition of the chromosomes. There is, however, some evidence that differences in the internal structure of the chromosomes may be responsible for differential X-ray susceptibility. NAVASHIN and his collaborators have found that the aging of seeds increases both the mutation rate and frequency of spontaneous chromosomal aberrations (cf. GILES 1940). OFFERMANN (1938) has shown that X-rays produce more mutations in old than in young sperm of Drosophila. These results suggest that aging of chromosomes induces internal changes, probably of a chemical nature, which makes them more susceptible to both spontaneous and induced alterations.

In any analysis of differential susceptibility of different stages in the nuclear cycle it is essential to differentiate the physiological effect of X-rays from the production of mutations and gross chromosomal aberrations which lead to genetic alterations. The physiological effect of suppressed nuclear activity and clumping of the chromosomes has long been known and this reaction in plant cells has been dealt with in considerable detail by MARQUARDT (1938). At moderate X-ray doses the inhibition of nuclear development is temporary and the cell recovers. The time of recovery depends on the dosage (CARLSON 1938, and unpublished). Heavy X-ray dosage may cause so much clumping of the metaphase chromosomes that normal division is prevented and the effect is lethal. Maximum X-ray effects at metaphase probably can be attributed to physiological factors.

It is probable that the physiological and genetic effects can be differentiated by determining the temperature coefficient of the reaction to X-rays. If chromosome aberrations are responsible for cell injury the temperature coefficient should be one or less than one, while if physiological effects are responsible the temperature coefficient should be appreciably greater than one.

The behavior of broken ends of chromosomes

The experimental evidence shows that the induced chromosomal aberrations involving two chromosomes are dependent upon two independent breaks limited in both time and space. The broken ends of chromosomes either reunite in the original position or produce chromosome aberrations within a short time after the breaks are induced by X-rays, except a very small proportion of breaks producing broken ends which appear to have

lost their capacity for fusion. Most of the unions occur within a few minutes, and at the end of about an hour all fusions are completed. This does not necessarily mean, however, that a broken end can remain in an unstable condition for only an hour.

STADLER (1932) has assumed that an acentric fragment may be included in a daughter nucleus by chance and fuse with the original broken chromosome after several cell generations. Such behavior is highly improbable for the following reasons: all potential fusions between broken ends are completed in about an hour in the Tradescantia microspore which has a nuclear cycle of about a week; nearly all the acentric fragments found at metaphase have no broken ends since the two ends of such fragments are the normal ends of chromosomes or chromatids; the few terminal deletions which persist until metaphase appear to have lost their capacity for fusion since chromosome fragments show no lateral fusion of sister chromatids; and very few of the various types of fragments are included in the mitotic daughter nuclei in plant cells. Acentric fragments of meiotic chromosomes often are included in the daughter nuclei in Zea (McCLINTOCK 1938b), and in the neuroblasts of the grasshopper such fragments usually are included in the daughter nuclei (CARLSON 1938). Even in these cases reunion of acentric fragments is improbable. Moreover, according to McCLINTOCK, the acentric fragments occasionally included in the Zea microspores act as normal chromosomes both in their cytological behavior and in their genetic influence. Thus there could be no genetic deficiency by deletion of a chromosome segment, or genetic recovery when the fragment joins the centric segment, as STADLER has postulated.

The temperature effect on induced chromosomal aberrations and mutation

According to MULLER (1935), PAPALASHVILLI has found that the frequency of chromosomal rearrangements in Drosophila is increased by raying the flies at low temperatures. MICKEY (1937) has found a much higher frequency of translocations in flies rayed at 15°C than in those rayed at 28°–34°C. In Tradescantia microspores both chromosome and chromatid aberrations, induced at either the resting or at mid-prophase, are much more frequent in the cells rayed at low temperatures. The variations in temperature affect both one-hit and two-hit aberrations. The decreased frequency of X-ray induced aberrations at the higher temperatures can be attributed to a more rapid fusion of broken ends or to a more relaxed condition of the chromosomes which inhibits chromosome movements. Either factor would favor the reunion of broken ends in the original position and decrease the frequency of illegitimate unions which produce the

aberrations. It has been shown that the frequency of the initial breaks is relatively independent of the temperature.

The reversed temperature effect on the chromatids at earliest prophase is difficult to explain. The reaction of the chromosomes cannot be due to changes in the nucleus as a whole because the single chromosomes show a decreased frequency of aberrations, and the split chromosomes an increased frequency of aberrations at high temperatures. An increase in the speed of union of broken ends may be involved. At earliest prophase the newly formed chromatids may be so closely associated that a break would separate the broken ends of a single chromatid further than the distance between chromatids. At mid-prophase the chromatids may be separated by a distance greater than the distance between broken ends of a chromatid. Rapid union of broken ends would tend to produce lateral fusions and consequently aberrations at early prophase, but would favor reunion in the original position and consequently fewer aberrations at later prophase stages. This interpretation could hardly be applied to the two-hit chromatid aberrations, which also seem to show the reversed temperature effect at early prophase, although the numbers are very low. An increase in torsional strain induced by high temperatures only at earliest prophase could also be invoked to explain these results, but further work is necessary in order to determine the factors involved in the reversed temperature effect at earliest prophase.

The nature of X-ray induced breaks

The chromosomal aberrations induced by X-rays have been attributed to fusions followed by breaks and to independent breaks followed by fusions between broken ends. The contact hypothesis sponsored by MULLER (1932) postulates that the chromosomes in contact or closely associated may be affected by a single X-ray hit. The breakage hypothesis, suggested by STADLER (1931), postulates that independent breaks are followed by illegitimate fusions. The analysis of X-ray induced chromosomal aberrations in Tradescantia confirms STADLER'S hypothesis. The dosage curves for one-hit and two-hit aberrations, the effect of the time-intensity factor, and the temperature effect, all prove that aberrations involving two chromosomes are dependent upon two independent hits.

The one-hit chromatid breaks appear to be caused by single X-ray "hits" since the relation between dosage and frequency of such aberrations is approximately linear. Since two sister chromatids may be broken by a single hit the ionization or excitation must cover a relatively large range because these sister chromatids are separated by a distance of at least a tenth of a micron at mid-prophase. The frequency of induced mutations in Drosophila is independent of X-ray wave-length (TIMOFÉEFF-RESSOV-

SKY 1936). The frequencies of X-ray induced chromosomal aberrations in Tradescantia also are independent of wave-length (RICK, unpublished). These results indicate that the X-ray effects are caused by ionization (GOODSPEED and UBER 1939).

The broken ends of chromosomes may remain in an unstable condition and capable of fusion for an hour or longer. It is surprising that a molecular complex of such a nature can remain in an unstable condition for so long a period. When fusions between broken ends do occur the union appears to be quite perfect in most cases, and breaks in subsequent chromosome bridges are no more likely to occur at the point of union than at other loci. There must be natural breaking points in the chromosome because breaks and fusions are normal features of practically all chromosomes at the time of meiosis. The reunion of both X-ray induced and crossover breaks appears to have no effect on the physical condition of the chromosome.

About five percent of the visible chromosome alterations induced by irradiation of the resting nucleus consist of terminal deletions. The broken ends of such deletions appear to behave as normal ends. Such a behavior may be caused by breaks at loci other than the natural breaking points.

ACKNOWLEDGMENTS

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SUMMARY

An analysis of X-ray induced chromosomal aberrations in the microspores of Tradescantia has led to the following conclusions:

1. The chromosomes react to X-rays as though they were single threads during the resting stage and become effectively split to form sister chromatids at early prophase.

2. The effective splitting of an individual chromosome occurs very rapidly, and there is some evidence that splitting begins at the region of the centromere and progresses distally.

3. The simple terminal deletions are readily induced at prophase because the two sister chromatids may be broken by one hit, followed by lateral fusion of broken ends of sister chromatids.

4. The frequency of these simple deletions shows an approximately linear relation to dosage. The equation for this relation is percent B $=(D/K)^{1.1}$, where B is the number of effective breaks in relation to the total number of chromosomes, D the dosage in r units, and K a constant.

5. A simple deletion induced at the resting stage persists until metaphase only when the broken ends lose their capacity for fusion. Such breaks are rare. The broken ends of these chromosomes appear to behave as normal ends.

6. Aberrations involving two chromosomes, or two loci in the same chromosome, are dependent upon two independent breaks, limited in both time and space.

7. The frequency of these two-hit aberrations is dependent upon the radiation intensity. High intensity is more effective than low intensity because the aberrations are dependent upon two adjacent breaks within certain limits of time. The frequency of one-hit aberrations at a given dosage is independent of the time-intensity factor.

8. The frequency of two-hit aberrations increases exponentially with increased dosage. When the dosage is varied by varying the time of exposure the relation between frequency of aberrations and dosage is percent $B=(D/K)^{1.5}$, but if dosage is varied by varying the intensity the equation is percent $B=(D/K)^{2.0}$.

9. Intermittent dosage experiments show that broken ends of chromosomes may remain in an unstable condition for as long as an hour before fusion, although most fusions occur in a much shorter time.

10. Only a small proportion of the X-ray induced breaks result in visible chromosome aberrations. Most of the broken ends reunite in the original positions with no evident alteration of the chromosome.

11. The frequency of the initial breaks induced by X-rays is relatively independent of temperature. The production of aberrations, however, is greater when the resting and prophase nuclei are irradiated at low temperatures. High temperatures during or immediately following irradiation increase the reunion of broken ends in the original position thus decreasing the frequency of aberrations. This temperature effect is reversed at very early prophase.

12. The initial breaks induced by X-rays must be distributed at random, but both the one-hit and two-hit aberrations occur more frequently at the proximal ends of the chromosome arms. This distribution of effective breaks is attributed to the effect of secondary factors.

13. Differential X-ray production of chromosomal aberrations in different phases of the nuclear cycle, in different cells of the same organism, and in similar cells of different organisms, can be attributed, in part, to secondary factors, such as proximity of chromosomes, freedom of chromosome movement, and the physical condition of the individual chromosome.

14. The X-ray induced breaks appear to occur usually at natural breaking points. Most of the breaks and fusions following irradiation appear to be comparable to those occurring naturally at the time of crossing over.

ADDENDUM

I have seen, in manuscript, a paper on X-rayed Tradescantia chromosomes submitted to "Genetics" in July 1939 by A. C. FABERGÉ of University College, London. FABERGÉ's conclusions on the time factor and the temperature effect are essentially the same as those reported above. His data were not adapted to an analysis of the dosage curve, and his consions in this regard appeared to require reconsideration.*

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SPONTANEOUS CHROMOSOME ABERRATIONS IN TRADESCANTIA

NORMAN GILES
Harvard University, Cambridge, Massachusetts

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THE occurrence of spontaneous chromosome aberrations has been observed in numerous genera of both plants and animals. These natural structural changes have consisted largely of reciprocal translocations and inversions, which when heterozygous result in characteristic configurations visible at meiosis. Similarly, such changes are evident in the salivary gland chromosomes of Drosophila. They also may be detected by genetic analysis where the linkage maps are adequate. Structural alterations of this nature may persist indefinitely, since they are usually viable in the haploid stage of the chromosome cycle. Many other types of structural changes must occur only to be lost because of lethal deficiencies. Spontaneous chromosome alterations are usually so rare that no adequate analysis can be made of the types and the frequency of their appearance. Further, it is seldom possible to determine the stage in the nuclear cycle at which the aberration occurred. In Tradescantia, however, a number of plants have shown a comparatively high frequency of spontaneous chromosome alterations, and the types of alterations found in the microspore chromosomes indicate their time of origin in the nuclear cycle.

In this analysis we have used several pure species of Tradescantia and the F_1 and F_2 plants from a natural species hybrid between *T. canaliculata* Raf. $\times$ *T. humilis* Rose. Smear preparations were made of the developing microspores at the first post-meiotic mitosis. The chromosomes were analysed for types and frequencies of natural chromosome aberrations. The nature of the spontaneous alterations suggested that natural radiation might be involved, and for comparative purposes the alterations induced by gamma rays and X-rays have been analysed.

TYPES AND FREQUENCIES OF X-RAY INDUCED AND SPONTANEOUS CHROMOSOME ABERRATIONS

The effects of X-rays on the chromosomes in the developing microspore of Tradescantia have been studied in considerable detail by several investigators (RILEY 1936; HUSTED 1936; MATHER 1937; SAX 1938; SAX and MATHER 1939). Their results have shown that the configurations produced by irradiation comprise two distinct classes, depending on whether the breaks and subsequent fusions occur before or after the effective splitting of the chromosomes. Breaks occurring when the chromosomes are

split are termed chromatid breaks, those when they are single, chromosome breaks. In the case of both classes, the breaks may involve only a single chromosome or may result in exchanges between two or more chromosomes. A study of the relation between types of breaks and dosage (SAX 1940) has shown that some configurations result from a single X-ray "hit" and some from two "hits." A single break involving only one of the two sister chromatids gives a shortened chromatid arm and an acentric fragment at the microspore division. If the hit breaks both arms, adjacent ends of sister chromatids fuse, and a dicentric chromatid and a U-shaped acentric fragment results. Chromatid exchanges involving two non-homologous chromosomes depend on two independent breaks. The types of chromosome breaks, which occur as a result of irradiation during the microspore resting stage, show similar relations to dosage. A single break in one chromosome results in a deletion; when the chromosome later divides the broken ends do not fuse—in sharp contrast to the behavior of ends of sister chromatids broken at the same locus—but give two shortened chromosomes and two rod-shaped acentric fragments at metaphase. Two breaks, one in each arm of the same chromosome, may reunite to form a ring chromosome. At anaphase, after splitting, separation of the ring may be free, interlocked, or as a single double size dicentric ring. Chromosome exchanges, involving two non-homologous chromosomes, also depend on two X-rays hits. Reunion of ends results either in a reciprocal interchange, which is usually equal in the case of Tradescantia, and hence not detectable, or in a dicentric chromosome and an acentric fragment. The dicentric divides normally, and at anaphase may separate freely, form two bridges, or interlock, depending on the amount of relational coiling between the centromeres. The fragment also divides normally, and produces two rod-shaped acentric fragments at metaphase.

A study of the relation between the frequency of the various types of chromosome and chromatid breaks and the X-ray dosage has led to the derivation of specific equations relating the percentage of breaks to the dosage in roentgens (SAX 1940). For one-hit chromatid breaks this equation is $B=(D/45)^{1.1}$, where B = percent breaks and D = dosage in roentgens (r). The single breaks evidently tend to increase directly with the dosage. For the two-hit chromatid exchange breaks, however, the equation is $B=(D/67)^{1.9}$, the increase in breaks being approximately as the square of the dosage. A comparison of actual counts on which these equations are based shows that the one-hit breaks are much more frequent at low dosages than the two-hit. For example, at 10r the frequency of one-hit chromatid breaks is .2 percent whereas that of the two-hit breaks is .03 percent. At 100r, however the frequencies are almost equal, the one-hit breaks equal 2.4 percent, and the two-hit 2.0 percent. The percentage of

dicentric and ring chromosomes at 10r is .01 percent, and at 100r is .8 percent.

A study of the position in the chromosome of X-ray induced breaks (SAX and MATHER 1939) has shown that they do not occur at random, but tend to be localized in the regions proximal to the centromere.

The types of chromosome aberrations found occurring spontaneously are very similar to those produced by irradiation. The most frequent is the chromatid break involving both sister chromatids at the same locus, and resulting in a dicentric chromatid bridge accompanied by a fused acentric fragment at anaphase (Plate I, figure A, B, C). Single chromatid deletions also occur, and one case of a chromatid exchange, producing a dicentric chromatid and an acentric fragment, was observed in a triploid (figure H). Chromsome breaks were observed less frequently than chromatid, and single chromosome deletions (figure F) are more common than exchange breaks involving two chromosomes. Aberrations which had occurred previous to or at the time of meiosis were noted occasionally. In this category at metaphase are included those chromosomes which have lost all or part of an arm and have no accompanying acentric fragment. They have been observed only in microspores containing at least a normal haploid complement. It is probable that the centric fragments which occur with considerable frequency in certain species of Tradescantia (WHITAKER 1936) are the result of such losses (figure G). A few unequal reciprocal translocations which had evidently occurred during the post-meiotic stages were also noted.

The chromosome configurations resulting from spontaneous structural changes during the post-meiotic development of the microspore must be carefully distinguished from somewhat similar types which may appear at the microspore mitosis as a result of chromosome behavior at meiosis. There is no difficulty in distinguishing single chromosome and chromatid breaks, producing shortened arms and acentric fragments, for these could only result from single breaks with no subsequent fusion of ends during the post-meiotic development of the microspore. In the case of bridge configurations, however, more care is necessary.

The principal types of chromosome bridge configurations which may occur at the first post-meiotic mitosis in pollen grains and their methods of origin are represented in figure 1. Most of these types have been recorded as occurring spontaneously or as a result of irradiation, and although some other types are also theoretically possible according to our present knowledge of chromosome behavior, they must be of extremely rare occurrence.

Dicentric chromosomes and chromosome bridges may result from chromosome behavior at meiosis or during the post meiotic development of the microspore. Crossing over in heterozygous inversions at meiosis may lead

to two distinct types of dicentrics at the succeeding pollen grain mitosis. Non-disjunction at second anaphase of a dicentric chromatid resulting from single crossing over within an inversion and disparate crossing over proximal to the inversion produces a dicentric chromosome which divides

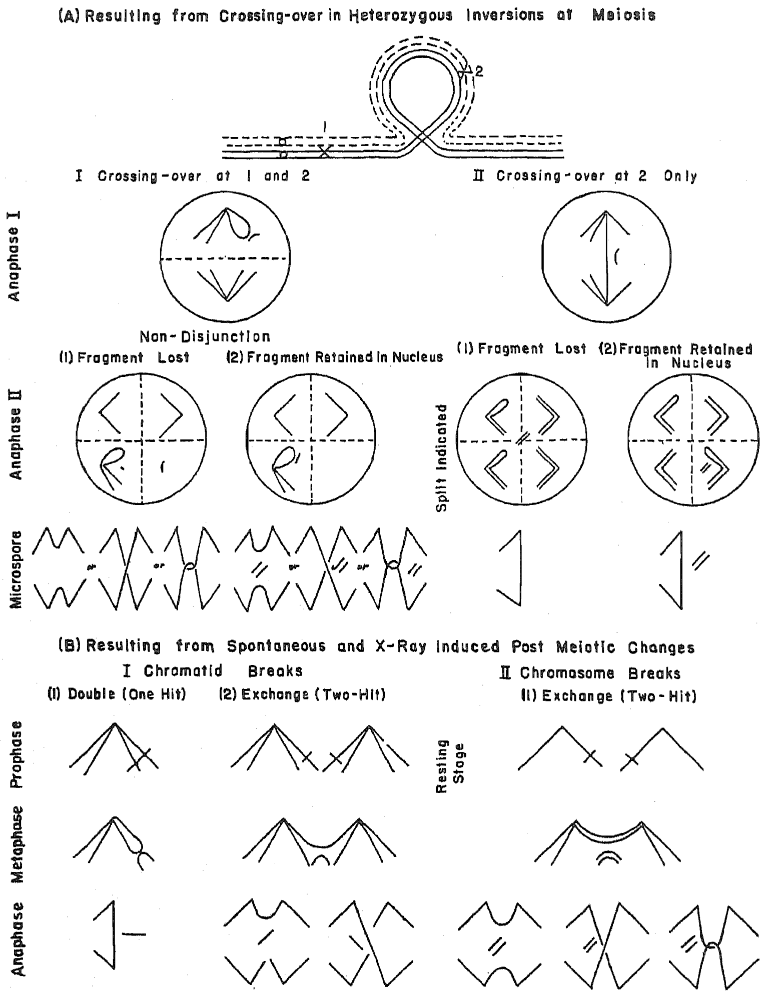

FIGURE 1.—Principal types and methods of origin of chromosome bridge configurations occurring at the first post-meiotic mitosis in microspores. (For discussion see text.)

mitotically and separates at anaphase of the pollen grain mitosis (figure 1; A, 1). The manner of separation depends on the amount of relational coiling between the centromeres, and may be free, criss-cross, or interlocked. This non-disjunction type has been noted in Rhoeo by DERMEN (EMSWELLER and JONES 1938), in a triploid Tulipa by UPCOTT (1937), and in a triploid Tradescantia (GILES, unpublished). Normally the resulting

acentric fragment is not included in the nucleus, but if included through both divisions it will divide and appear as two acentric rods at metaphase. The suggestion has been made by EMSWELLER and JONES (1938) that such bridge configurations may also result, particularly in hybrids, from the distribution of dicentrics formed as a result of crossing over in the region between the centromeres of chromosomes paired in such a manner that their centromeres are not opposite each other. The second type of dicentric produced at the microspore mitosis results from the behavior of broken ends of chromatids following the breakage of an inversion bridge due to a single crossover within an inversion (figure 1; A, II). In this case, the broken ends of the inversion bridge either fail to divide normally during the succeeding resting stage, or, if the chromosome is considered as already split, the ends of sister-half chromatids fuse. The result in the case of either interpretation is a dicentric chromatid. The resulting single bridge of this type was described by SAX (1937) in a triploid Tradescantia, and in Zea by MCCLINTOCK (1938). In many plants the acentric fragment produced by the inversion crossover is lost in the cytoplasm during meiosis. In Zea however, it is sometimes included within the nucleus through both meiotic divisions, and in such cases, after division during the resting stage, two rod-shaped fragments are visible at the time of the pollen grain mitosis.

The types of bridges resulting from aberrations occurring during the post-meiotic development of the microspore have already been described. The single chromatid dicentrics produced by breakage and fusion between sister chromatids at the same locus are accompanied by single acentric fragments which distinguish them from those cases in which the acentric fragment is included in the nucleus with a broken bridge at meiosis. Single dicentrics resulting from chromatid exchanges may pass to one pole or form a bridge; single acentric fragments are present in these cases also (figure 1; B, I). Chromosome exchanges produce dicentrics which give the same configurations as do the meiotic non-disjunction dicentrics. In this type, however, two rod-shaped acentric fragments are normally always present (figure 1; B, II).

The occurrence of single chromatid bridges at anaphase of the microspore division in a triploid Tradescantia and a diploid Hyacinthus has been noted by UPCOTT (1937) and attributed to post-meiotic spontaneous structural changes in the chromosomes. No distinction is made, however, between the types of bridges noted. As in the case of the Tradescantia, those bridges accompanied by single acentric fragments were evidently due to the changes inferred. The bridges without fragments, however, presumably resulted from the breakage of inversion bridges at meiosis. In the Hyacinthus, few fragments were observed, and since the bridges appeared to be about equal in length to the sum of the two free arms—the

chromosome involved had a median centromere—the conclusion is drawn that normal chromosome ends have fused. This observation does not agree with most of the recent X-ray evidence, which has supported the idea that normal ends have properties not possessed by interstitial loci. It seems much more probable that such bridges without fragments are also the result of breakage of inversion bridges at meiosis. The length of the bridge could be accounted for if the inversion were very near the end of the chromosome. Such a condition would also favor the occurrence of bridges at the microspore division, for the small deletion resulting from the crossover would quite possibly not be sufficient to prevent the post-meiotic development.

Observations on the occurrence of spontaneous chromosome aberrations observable at the pollen tube mitosis in Paeonia and Kniphofia by BARBER (1938) are also interpreted as demonstrating the fusion of homologous ends of sister chromatids. Although no particular study was made at this stage in Tradescantia, the configurations observed lend no support to the above conclusion. Only bridges with acentric fragments were noted (Plate I, E). The pollen used was not subjected to controlled aging, however, as was the case in the study cited. It is possible that aging of pollen may produce results similar to the initial effects of heat and X-rays which cause terminal fusions, apparently involving the chromosome envelope (SAX 1938).

In the diploid plants of Tradescantia examined in the present study no bridges at the microspore mitosis without fragments were noted. The absence of single bridges without fragments apparently shows that even small deletions are sufficiently lethal in the haploid condition to prevent the development of the microspore. It is possible, however, that further

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DESCRIPTION OF PLATE I

Photographs of aceto-carmine smear preparations showing spontaneous chromosome aberrations in Tradescantia. Ca. 1200×.

FIGURE A.—Microspore anaphase in diploid. Dicentric chromatid and fused acentric fragment, upper right.

FIGURE B.—Microspore anaphase in diploid. Dicentric chromatid and fused acentric fragment at left.

FIGURE C.—Microspore anaphase in diploid showing seven chromosomes as a result of non-disjunction. Stretched bridge of dicentric chromatid, with long acentric fragment above.

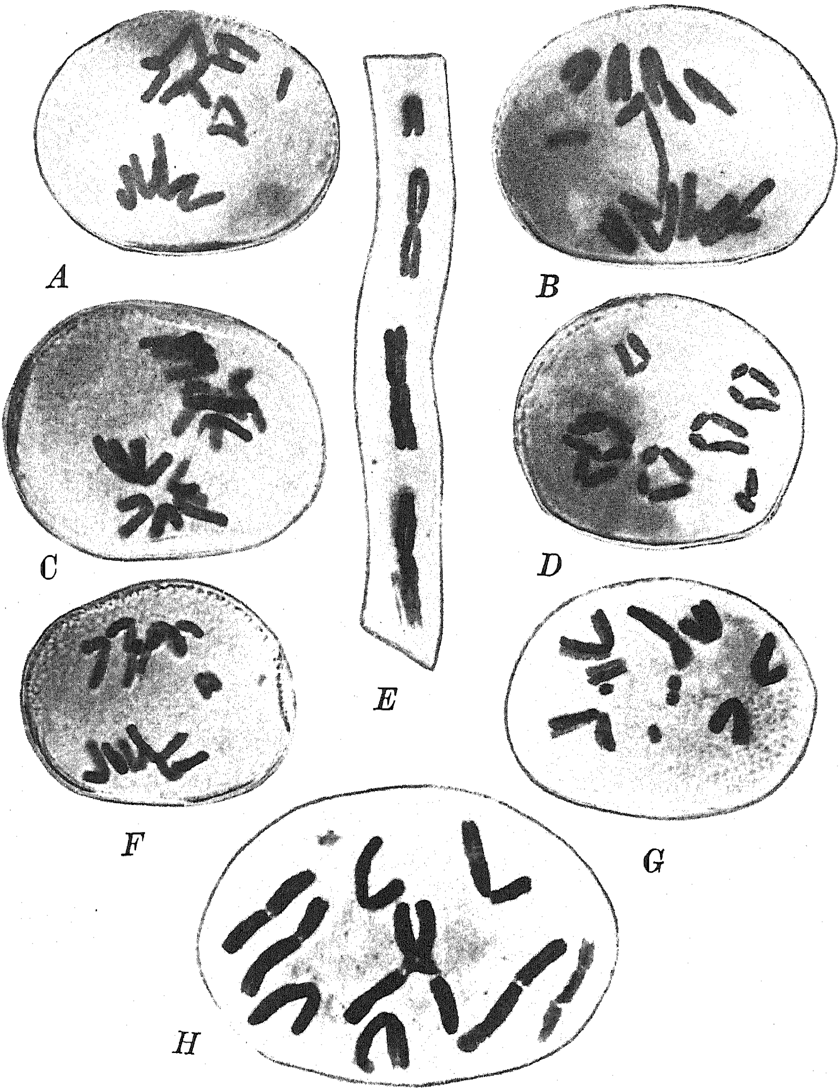
FIGURE D.—Microspore early anaphase in diploid. Dicentric chromatid. Two acentric fragments at lower right, apparently resulting from failure of fusion of ends.

FIGURE E.—Pollen-tube division in diploid, only three chromosomes shown. Dicentric chromatid and U-shaped acentric fragment at top.

FIGURE F.—Microspore anaphase in diploid. Single chromosome break showing divided acentric fragments. Shortened chromosomes at poles.

FIGURE G.—Microspore metaphase in diploid. Extra centric arm and four centric fragments present.

FIGURE H.—Microspore metaphase in triploid. Chromatid exchange break.


A
B
C
D
E
F
G
H

study will reveal that this is not always the case. In only one case was a chromatid bridge with two fragments observed (Plate I, D). A study of this figure indicates that it is probably due to the failure of fusion of the distal ends of sister chromatids following a double chromatid break. Similar behavior has been noted very rarely in the case of X-ray induced double chromatid breaks. Observations at meiosis indicate that acentric fragments resulting from inversion crossovers are not included in the nucleus in Tradescantia, and the almost complete absence of single bridges with divided fragments substantiates this. In triploids, single bridges both with and without single fragments have been found, but no cases of bridges with double fragments have been seen, again indicating that fragments at meiosis are usually lost in the cytoplasm.

In all the cases of chromosome dicentrics observed, two rod-shaped fragments were plainly visible. Although it is theoretically possible, as indicated in figure 1, that such configurations might be the result of non-disjunction of a dicentric chromosome at meiosis with the fragment being included in the nucleus, this is highly improbable. All the evidence indicates that a fragment is normally lost in Tradescantia and the chances of its being retained in the nucleus with the dicentric through both divisions at meiosis, as would be necessary to give a configuration similar to those observed, is negligible.

There is good evidence from recent X-ray experiments designed to determine the time of chromosome division that splitting occurs during the early prophase preceding the first post-meiotic mitosis in the microspore (MATHER 1937; SAX and MATHER 1939). Although it is not possible to determine the time of occurrence previous to the microspore mitosis of the various types of spontaneous aberrations, the fact that both chromatid and chromosome breaks occur and are not the result of natural radiation, as will be shown later, supports the X-ray evidence. It indicates that the kind of breakage resulting from irradiation is not due to any peculiar effect of the ionization itself, as has been suggested. However, the behavior of broken ends of chromatid bridges broken at meiosis appears not to support the X-ray evidence, for the resulting bridge formation can be most easily understood if the chromatid is considered to be already divided at meiosis. On the other hand, if the chromosome entering the resting stage is considered to be already split, the types of spontaneous breaks and the behavior of X-ray induced breaks are not easily explained. It seems possible that the behavior of the ends following bridge breakage may not be the same as in the case of X-ray induced and spontaneous post-meiotic breaks. The bridge chromosome apparently breaks under tension, or perhaps as a result of cytoplasmic enzyme action as suggested by EMSWELLER and JONES (1938), and it is possible that the resulting

damage at the locus of breaking may inhibit the later splitting of the chromosome at the region of the newly formed ends. Such a failure of division at the ends would result in a single dicentric bridge at the following mitosis. Single dicentric bridges without fragments would also result if the broken ends of meiotic bridges retained their ability to fuse until after splitting had occurred. The X-ray evidence indicates, however, that broken ends retain this ability for only about an hour after breakage (SAX 1939).

The single chromosome deletions, as well as the chromatid deletions, are of particular interest since they indicate that new stable ends may arise as a result of spontaneous breaks. Most of the evidence on chromosome aberrations shows that broken ends tend to fuse, and it has been suggested that permanent changes in the chromosome morphology cannot be effected

TABLE 1

Loci of spontaneous chromatid and chromosome breaks. Each locus includes one-fifth of chromosome arm, starting with locus one proximal to centromere and extending distally to end of arm.

LOCUS	CHROMATID N	CHROMATID %	CHROMOSOME N	CHROMOSOME %
1	24	50	10	36
2	7	15	11	39
3	8	17	5	18
4	5	10	2	7
5	4	8	0	0
Total	48		28	
Ave. Locus	2.0		1.1	

by terminal deletions. However, it now seems clear that stable ends may arise with a fair frequency as a result of both X-ray induced and spontaneous terminal deletions.

A study of the loci of the spontaneous breaks with respect to the centromere was made. To do this the various arms were divided into five loci measuring from the centromere to the ends. The relative positions of the breaks rather than the actual distance were used since the arms vary somewhat in length, and the individual chromosomes cannot be differentiated. The data are presented in table 1. It is evident that, as in the case of the X-ray induced breaks, the distribution of the observed aberrations is not at random. For chromatid breaks the average locus of the breaks is 2.0, and for chromosome breaks it is 1.1. This indicates a distinct localization in the region proximal to the centromere.

The relative frequencies of the different kinds of aberrations occurring spontaneously are also very similar to those obtained from irradiation at low dosages. The results of the present study are indicated in the totals to table 2. In the 78,021 chromosomes examined to determine the fre-

quency of the various types of alterations, there were 55 chromatid breaks giving dicentric chromatids, 26 chromosome deletions, and 2 dicentric chromosomes. Dicentric chromatids are therefore about twice as frequent

TABLE 2

Frequency of various types of spontaneous chromosome aberrations. Examples from hybrid F_2 to indicate variation. Totals of all plant groups.

PLANT	DATE	TOTAL CHROMOSOMES	ONE-HIT: DICENTRIC CHROMATIDS	%	ONE-HIT: CHROMOSOME DELETIONS	%	TWO-HIT: DICENTRIC CHROMOSOMES	%	TOTAL BREAKS	%
F_1										
Gentilly 5		2868								
Gentilly 13		1434	2	.14					2	.14
F_2 125	2/27/39	1956	4	.20	2	.10			6	.30
	3/ 3	1392								
	3/20	1260								
	5/ 8	1146	2	.17					2	.17
346	11/ 5/38	1290			1	.08			1	.08
	11/ 6	1326	1	.07					1	.07
	11/ 7	1327*								
	11/ 9	1296								
	11/10	1290								
	11/11	1262								
93	2/21/39	762	2	.26					2	.26
	2/26	1362	2	.15					2	.15
	3/ 1	1344	3	.22					3	.22
	3/ 1	546	1	.18					1	.18
	3/22	816			2	.24			2	.24
35	10/ 6/38	1821	4	.22	2	.11			6	.33
	2/15/39	1248	1	.08	2	.16			3	.24
	2/18	1260	1	.08					1	.08
	3/ 5	1362	4	.30					4	.30
Total F_2 (21 plants)		70,447	54	.076	26	.037	1	.001	81	.12
Total non-hybrid plants		7,574	1	.02	—	—	1	.02	2	.04
Total all plants		78,021	55	.070	26	.033	2	.003	83	.11

* Totals not multiples of six indicate occurrence of microspores with seven chromosomes as a result of non-disjunction at meiosis.

as chromosome deletions, and about thirty times as frequent as dicentric chromosomes. It will be recalled that at low irradiation dosages, one-hit chromatid breaks giving dicentric chromatids are much more frequent

than both chromatid and chromosome two-hit breaks. The ratio of single chromatid breaks to chromatid exchange breaks is of the order of 10 to 1; that of single chromatid to chromosome exchanges is about 20 to 1. Although there is no very adequate X-ray data for the one-hit chromosome deletions, the frequency of these types is evidently very much lower than that of the one-hit chromatid breaks involving both chromatids at the same locus. This is to be expected, as it seems clear that many more breaks occur as a result of irradiation than appear as visible aberrations following fusions in new associations. Single breaks occurring when the chromosome is single are very likely to heal back in the same position, and are thus not detected. Only a few fail to heal and lead to visible deletions. When a single hit breaks both chromatids of a chromosome at the same locus after division, however, the subsequent fusion of the adjacent broken ends produces a dicentric chromatid and a U-shaped acentric fragment, and the aberration is detected at the succeeding division.

The comparative frequencies of single chromosome deletions and one-hit chromatid breaks indicate that the former occur with a considerably greater relative frequency spontaneously than when X-ray induced. This seems to be due to the fact that the period during which chromosome deletions may occur lasts for from eight to ten days whereas that during which single chromatid breaks may originate is approximately 24 hours in length.

Single breaks occurring in one chromatid of a chromosome were not included in the tabulation since their frequency is extremely difficult to record accurately. As in the case of observations following irradiation, the degree of breakage may vary from achromatic lesions through partially broken ends to complete deletions.

SPONTANEOUS CHROMOSOME ABERRATIONS AND NATURAL RADIATION

The similarity between the types, the loci, and the frequency of chromosome aberrations occurring spontaneously and those produced by irradiation at low dosages suggested the possibility that the former might be caused by natural radiation. If this is the case, increasing the amount of irradiation above that to which the plants are normally subjected due to natural radiation (taken as 50 ions/cc/sec = .0022 r/day) should result in an increased frequency of breaks. In order to make this test at the low dosages involved, a radium capsule of known ionizing strength (5.4×10^{-6} r/min at 50 cm) was used as a gamma ray source. By placing this at various distances from the plants in accord with the inverse square law, it was possible to vary the irradiation intensity as compared to natural radiation. Two plants were tested, #393, which had shown a fairly constant frequency of spontaneous aberrations (table 2) and #404, in which

no aberrations had been noted. Double chromatid breaks, which are classed as one-hit chromatid breaks and result in a single bridge and an acentric fragment at anaphase, were used in the calculations for several reasons. They occur more frequently than any other type, and hence a better measure of their frequency can be obtained. Then, too, the period during which this type of break may originate is accurately known from the X-ray evidence, and consequently the amount of natural radiation to which the plant is subjected can be ascertained. Chromatid breaks are observed in the microspore within five hours after irradiation, and reach a distinct frequency peak at about 24 hours. The frequency then declines rather rapidly, and no chromatid breaks are found after 70 hours during the winter months. The average period during which the majority of breaks occur seems to be approximately 24 hours in length. It is also better to use one-

TABLE 3

Test by use of radium capsule of rôle of natural radiation in the production of spontaneous chromosome aberrations.

DISTANCE OF CAPSULE (CM)	TIME	APPROXIMATE AMOUNT BY WHICH NATURAL RADIATION IS INCREASED	#393			#404		
			TOTAL CHROMOSOMES	DICENTRIC CHROMATIDS (ONE-HIT)	%	TOTAL CHROMOSOMES	DICENTRIC CHROMATIDS (ONE-HIT)	%
30	24 hrs.	10	2329	3	.13	1254	—	—
3	24 hrs.	1000	1344	3	.22	1350	—	—
Control			1578	4	.26	1344	—	—

hit chromatid breaks rather than exchange breaks since it has been shown by SAX (1939) that this type of break is independent of the time factor. Consequently, even though they may occur at any time during the 24 hour period, their spontaneous frequency may be compared with the frequency of similar breaks induced by irradiation of known dosage acting for a short or long interval. The value used to represent the intensity of natural radiation, 50 ions/cc/sec, is that given by TIMOFÉEFF-RESSOVSKY (1931). The data are presented in table 3.

The amount of ionization produced by the capsule at a distance of 3 cm over a 24 hour period is approximately 2.2 r. Although this intensity would be expected to produce only about .05 percent aberrations, it is 1000 times that due to natural radiation. Even with this great increase in intensity, there was no increase in the number of aberrations.

Further evidence that natural radiation is not sufficient to account for the observed frequency of spontaneous breaks is obtained by comparing the actual frequency of these breaks with the expected frequency if ioniza-

tion due to natural radiation causes the breaks. As has been noted, the chromatid breaks studied depend on one X-ray hit and show an approximately linear increase with the dosage. For Tradescantia the equation relating the frequency of breaks to the dosage in roentgens is $B=(D/45)^{1.1}$. Using this equation, the percentage of breaks produced by a given X-ray dosage (for example, 100 r) and that which would be produced by a dosage equal to the intensity of natural radiation can be calculated and compared. The same value as before is used to represent the intensity of natural radiation. The frequency of one-hit spontaneous aberrations, .07 percent, is the percentage of breaks recorded in the total of 78,021 chromosomes of both hybrid and non-hybrid plants examined (table 3). The calculations are outlined as follows:

1. Equation for one-hit (double chromatid breaks): percent $B=(\text{Dosage in r}/45)^{1.1}$
2. Percent B at 100 r = 2.4 percent.
3. Amount of natural radiation = 50 ions/cc/sec.
4. Period during which double chromatid breaks occur = 24 hours = 8.6×10^4 sec.
5. $1\ \text{r}=2\times 10^9$ ions/cc.
6. Amount of natural radiation occurring during the 24 hour period =

$$\frac{50\times 8.6\times 10^4}{2\times 10^9}=.0022\text{r}.$$

7. Percent of one-hit breaks occurring spontaneously = .07.
8. Amount of radiation necessary to produce .07 percent B = 4.0 r.
9. $\therefore$ 4.0 r/.0022r = ca 1818 — the amount by which the natural radiation intensity is too small to account for the observed spontaneous break frequency.

The value given by TIMOFÉEFF-RESSOVSKY, 50 ions/cc/sec, and used in the present calculations for the amount of ionization due to natural radiation probably represents a maximum. MULLER and MOTT-SMITH (1930) used 30, and BLACKETT (1935) gives the value as between 20 and 30 ions/cc/sec. The use of the higher value tends to keep the result indicative of the amount by which the intensity of natural radiation is too small to account for the rate of spontaneous chromosome aberrations on the conservative side. However, it is also possible that a longer period should be allowed for the action of natural radiation in the production of chromatid breaks during the period preceding the microspore division. This would tend to lower the calculated value.

The actual frequency of all one-hit spontaneous breaks occurring during the 24 hour period is undoubtedly higher than .07 percent. Single chromatid deletions were not recorded, and neither inversions, unless they involve

a considerable change in the position of the centromere, nor small ring or rod deletions can be detected at the microspore metaphase. It has been shown by HUSTED (1937) that structural changes may occur with some frequency during the development of the microspore of *Paris quadrifolia* and *Pancratium illyricum* without abnormalities being found at metaphase or anaphase. It is perfectly legitimate to use this percentage in the preceding calculations, however, since the X-ray equation in which it is used is also based on counts of one-hit chromatid dicentrics only.

Since one of the plants tested with the radium capsule, #393, showed a rather high and fairly constant rate of spontaneous chromosome alterations while the other, #404 showed none at all, it was decided to test the sensitivity of the two plants to the same dosage of X-rays. It might be expected *a priori* that the plant showing the higher percentage of spontaneous breaks would have a lower threshold of response and be more sensitive to X-rays, especially if natural radiation were the cause of the

TABLE 4

Sensitivity to X-rays. 320 r. Examined for chromosome breaks five days after raying.

PLANT NO.	TOTAL CHROMOSOMES	NO. OF BREAKS	PERCENT
393	4062	408	10.4
404	4566	502	10.9

breaks. Inflorescences of both plants were rayed at the same time and received a dosage of 320 r (80 r/min for 4 min). The buds were examined after five days for chromosome breaks. Three separate tests were made at different times, and the combined data are presented in table 4.

The results show that the two plants are equally sensitive to the same dosage of X-rays and may be taken as further support for the conclusion that natural radiation is very rarely involved in the production of spontaneous chromosome aberrations. The failure to find an increased sensitivity to X-rays in the plant showing a high frequency of spontaneous chromosomal aberrations is somewhat similar to the effect of irradiation on the rate of change in unstable genes of *Drosophila virilis* (DEMEREC 1934). In the latter case, however, an X-ray dosage which would increase the general mutation rate by about one thousand percent did not produce a significant increase in the rate of change of the unstable gene tested. In the present study, although there was no differential sensitivity, the rate of chromosome aberrations was increased by the X-ray dosage by about the expected amount. This is to be expected since the chromosome aberrations are not reversible reactions as has been postulated to be the case for the unstable gene mutations.

The evidence that the rate of spontaneous chromosome aberrations in Tradescantia cannot be accounted for in terms of natural radiation agrees with the results obtained for natural mutations in Drosophila. When it was first demonstrated by MULLER that irradiation produces a marked increase in the mutation frequency in Drosophila, it was at once suggested that natural radiation might be the cause of spontaneous mutations. Early discussions and experiments by OLSON and LEWIS (1928), BABCOCK and COLLINS (1929), and HANSON and HEYS (1930) appeared to indicate that this might be the case. It was soon shown, however, by the calculations and experiments of MULLER and MOTT-SMITH (1930) and TIMOFÉEFF-RESSOVSKY (1931) that the amount of ionization due to natural radioactive substances is much too small to account for the natural mutation frequency. The results obtained by MULLER and MOTT-SMITH indicated that the natural mutation frequency in Drosophila is at least 1300 times as high as it would be if it were caused solely by natural radiation; those of TIMOFÉEFF-RESSOVSKY, that it was at least 500 times as high. Later, the possible rôle of cosmic rays in causing mutations was suggested by THOMAS (1936) and others. But in this case, too, it has been fairly clearly shown by the calculations of DELBRÜCK and TIMOFÉEFF-RESSOVSKY (1936) and the experiments of JOLLOS (1937, 1939) that the amount of this type of ionizing radiations is also much too small to account for the natural mutation frequency.

NATURE OF SPONTANEOUS CHROMOSOME ABERRATIONS

It has been noted that the localization of spontaneous breaks in the proximal region of the chromosomes agrees with the findings in the case of X-ray induced breaks (SAX 1938; SAX and MATHER 1939). As pointed out in the latter paper "the initial breaks induced by X-rays should be distributed at random. There is good evidence that most of the broken ends reunite in the original association and that the permanent aberrations are associated with tortional strains which inhibit normal reunion of broken ends of chromatids and chromosomes . . . . The localization of breakage is attributed to the existence of a greater tortional stress in the proximal regions." In the case of the spontaneous aberrations, however, there is no obvious reason to assume a random distribution of breaks. Consequently, the localization of the breakage in these cases suggests that the tortional strains may be directly responsible for the breaks, not secondary factors as in the case of the X-ray induced aberrations. The much higher frequency of alterations occurring after the chromosomes are split into chromatids than when they are unsplit appears to be correlated with the difference in the nature and amount of coiling at these two stages. During the resting stage only relic coiling is present, whereas during the prophase

the sister chromatids are relationally coiled as well. The X-ray evidence also supports this conclusion since sensitivity of the chromosomes, as measured by the frequency of breaks reuniting in new associations, is greater during the prophase, when both types of coiling are present, than during the resting stage. It has been demonstrated that seedlings grown from aged seeds show a pronounced increase in the frequency of spontaneous chromosome structural changes (NAVASHIN 1933, NAVASHIN and GERASSIMOVA 1936, PETO 1933). As has been pointed out, this evidence suggests that the physiological conditions attendant on aging may be the cause of the increase in chromosome alterations. If chromosome structural mutations in general are the result of physiological conditions within the cell, not affecting the chromosome coiling mechanism, the loci of such breaks might be at random. In such a case the localization of the breakage in the regions proximal to the centromere would then be considered a secondary effect, as in the case of X-ray induced breaks.

The frequency of spontaneous structural changes of the types studied varied with the individual plants used. Also, some plants were found that showed a fairly constant percentage of aberrations whenever examined, while others varied considerably from one observation to the next. Some selected examples, presented in table 2, illustrate these points. There was no evidence of unusual meiotic behavior in individuals of the hybrid population showing a constant and high frequency of chromosome aberrations at the microspore mitosis. The chiasma frequency, percent of inversion bridges, and chromosome distribution were approximately the same as in the plants showing few or no spontaneous structural changes. There is some indication, however, that the difference between the plants of the F_2 generation may have a genetic basis. One of the F_1 parents showed no breaks in 2868 chromosomes examined, while the other showed two breaks in 1434 chromosomes, or .14 percent. The percentage of breaks in the F_2 segregates varied considerably. Some plants were found with frequencies considerably higher than the average for the F_2 of .12 percent. The highest frequency recorded was .48 percent. Other plants had a very low frequency, some showing no breaks in any of the chromosomes examined. Similar evidence that spontaneous structural chromosome changes may be genetically controlled is indicated by the "sticky chromosome" mutant of Zea (BEADLE 1932) and the variation in frequency of aberrations in the F_1 progeny of X-ray treated plants of *Crepis capillaris* (LEVITSKIJ 1937). The presence of genetic factors affecting the frequency of both visible and lethal spontaneous gene mutations in *Drosophila melanogaster* has been reported by DEMEREC (1937). Here, however, the genetic tests indicated that the spontaneous lethals were not connected with chromosomal aberrations.

The frequency of spontaneous breaks in the hybrid plants was about three times as high as in the pure species examined. It is well known that hybridization often results in irregularities in chromosome behavior which may produce changes in the number and morphology of the chromosomes (NAVASHIN 1927, 1934, MÜNTZING 1934, EMSWELLER and JONES 1938). These changes are usually associated with the mode of pairing and crossing over at meiosis in the hybrid, but there is also evidence for their spontaneous occurrence in somatic cells. In the case of some Triticum-Secale hybrids (PLOTNIKOWA 1932) the frequent occurrence of chromatid bridges and fragments at anaphase in root-tip mitoses was noted. Here, too, there was considerable variation in the frequency of bridge formation among the individuals of the same filial generations. It seems possible that the genetic recombination resulting from hybridization may be the cause of the higher percentage of visible structural chromosome aberrations, perhaps by disturbing the normal timing of the cycle of chromosomal coiling.

The occurrence of a high frequency of spontaneous chromosome aberrations in hybrids may perhaps be of significance in species formation, as it now seems clear from numerous studies that specific differences in both plants and animals are quite often associated with chromosome rearrangements. In the genus Tradescantia, hybridization under natural conditions is quite frequent (ANDERSON 1936, 1938) and this should provide opportunity for an increased number of structural chromosome changes.

Recently, a high mutation frequency has been noted in hybrids between races A and B of *Drosophila pseudoobscura* by STURTEVANT (1939). It is stated that there is no indication of an increase in major chromosome aberrations such as accompany X-ray treatment. Small rearrangements have not been investigated, however. It is still quite possible that chromosome aberrations of the type reported in the present Tradescantia hybrids may occur with some frequency in the Drosophila hybrid. Single chromatid dicentrics, which constitute the majority of the breaks found in Tradescantia, would not be viable, and the present method fails to indicate small inversions and deletions. These latter aberrations apparently show a linear increase with X-ray dosage, as do mutations, and such aberrations may be the cause of many if not most of the visible and lethal mutations. Evidence of a correlation between the occurrence of mutations and chromosome rearrangements is indicated by the work on Sciara by METZ and BOCHE (1939). In the progeny of X-rayed virgin female flies there is a very low incidence of both mutations and chromosome rearrangements. On the other hand, a high frequency of both rearrangements and mutations occurs as a result of X-raying sperm. The evidence from the present study that natural radiation is insufficient to account for the frequency

of spontaneous chromosome aberrations establishes a further similarity between mutations and chromosome aberrations.

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SUMMARY

The types, frequency, and loci of spontaneous structural chromosome aberrations in a number of pure species and in the F_2 generation of a natural species hybrid of Tradescantia have been studied at the first post-meiotic mitosis. These aberrations are similar in most respects to chromosome alterations induced by low dosage X-rays in the developing microspore. This similarity suggested that the spontaneous breaks might be due to natural radiation. Considerable evidence is presented, however, to show that this is not the case. By employing a radium capsule, the plants were subjected to irradiation 1000 times that due to natural radiation, but no increase in aberrations was found. Using X-ray data relating percentage of breaks to dosage, the dosage required to account for the observed frequency of spontaneous breaks was calculated. The amount of natural radiation was found to be approximately 1800 times too small to account for this frequency. Plants showing a considerable difference in the percentage of spontaneous breaks showed no differential sensitivity to the same X-ray dosage.

The localization of the breaks in the region of the chromosome arms proximal to the centromere, and the greater frequency of chromatid as compared with chromosome breaks suggests that the tortional strains associated with relic and relational coiling during the chromosome cycle may be a major factor in the causation of spontaneous breaks.

The frequency of spontaneous aberrations was found to vary widely among the individuals of the F_2 generation of the hybrid, and evidence is presented suggesting the genotypic control of spontaneous chromosome structural aberrations.

The average percentage of breaks in the hybrids was about three times that of the pure species. It is suggested that this increase in aberrations may result from the recombination of genetic factors following hybridization.

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THE INHERITANCE OF INTERGENERIC DIFFERENCES IN ZEA-EUCHLAENA HYBRIDS[1]

D. G. LANGHAM
Cornell University, Ithaca, New York

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INTRODUCTION

IN THIS study of Zea-Euchlaena hybrids and their segregating populations, all those characters (such as width of leaves and number of tillers) which show wide variation in both maize and teosinte were ignored and only characteristic differences were considered. Large populations of F_2 and backcross plants were grown to study the segregation of weak versus strong response to length of day, paired versus single female spikelets, many-ranked versus two-ranked ear, and many-ranked versus two-ranked central branch of the tassel. A brief abstract of this work has been published (LANGHAM 1939).

PHOTOPERIODISM

Weak versus strong response to length of day

The extensive research of GARNER and ALLARD (1920, 1923) has shown that length of day is of primary importance in determining the time of flowering of plants. Reproductive processes are initiated in some plants, such as red clover, iris, goldenrod and spinach by a relatively long day, while other plants, such as cosmos, soybean, violet and poinsettia require a relatively short day for flowering and fruiting.

EMERSON (1924) has shown that Durango teosinte, *Euchlaena mexicana*, is a member of the latter group; it is a short-day plant. If grown under long-day conditions it will remain in the vegetative state throughout the year. It can be forced into flowering in any season of the year, however, by exposing it for 30 days to short-day conditions (13 hours or less of daylight in each 24-hour period). If planted in Ithaca about May 15th, Durango teosinte will shed pollen early in October, because the 13-hour day begins about September 1st.

Maize of medium maturity planted in Ithaca about May 15th sheds pollen the first part of August. This difference of two months (August to October) in the flowering habits of maize and teosinte, and the fact that the hybrid between the two genera is highly fertile, suggested the possibility of studying the mode of inheritance of photoperiodism in maize-teosinte hybrids.

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Accordingly, Durango teosinte was forced into flower by "short-day treatment" in August 1936, and crossed with maize. The F_1 plants were grown in the greenhouse during the winter of 1936–37. These hybrid plants flowered about the same time as ordinary maize grown in the same greenhouse. F_2 and backcross seed was obtained and planted in the field May 22, 1937, for a preliminary study of the segregation for photoperiod. From the time the first plant shed pollen (August 3rd) until the first killing frost (October 4th) the plants were examined daily and the date of anthesis of each plant was recorded.

The distribution of the dates of anthesis in the backcross to maize is a unimodal curve with the greatest number of plants beginning to shed pollen between August 8th and August 12th. The backcross to teosinte gives

TABLE I

Date of anthesis and frequency of F_3 plants, 1938.

F_2 PLANT NUMBER 1937	DATE OF ANTHESIS IN 1937	AUGUST				SEPTEMBER						OCTOBER	
		13–17	18–22	23–27	28–1	2–6	7–11	12–16	17–21	22–26	27–1	2–6	7–
3	10/ 2												53
4	9/30												69
9	8/13				1	5	4	0	1	2	1	2	2
8	8/12		1	3	8	14	12	4	6	5	1	4	4
6	8/ 5	1	2	4	4	1	1						
10	8/ 4	1	2	8	3	4	4	2	0	1	1	0	1
2	8/ 3		14	9	7	9	7	1	2	2	1	1	1

a bimodal curve with one mode in August and the other in September. The F_2 also gives a bimodal curve, but the first mode is of much greater magnitude than the second. These F_2 and backcross distributions would be expected if the late-flowering habit of teosinte were determined by a single recessive genetic factor. This response to short-day in teosinte is hereby given the symbol *sd sd* and its allele in maize is designated by *Sd Sd*.

This unifactorial hypothesis was tested further by selfing some F_2 plants and growing their F_3 progeny under similar field conditions the next year. The two modes in the F_2 distribution can be represented by (1 *Sd Sd*+ 2 *Sd sd*) as the major mode, and (1 *sd sd*) as the minor mode. The chances are 1:2 that any F_2 plant taken from the major mode will be homozygous for the maize character, and the chances are 1:0 that an F_2 plant from the minor mode will be homozygous for the teosinte response to short day.

Table 1 shows that F_2 plants numbers 3 and 4, which were in the minor mode in 1937, gave, on selfing, a total of 122 late-flowering plants. This

indicates that these plants were homozygous for the teosinte response to short day, *sd sd*. F_2 plant number 6 gave a unimodal F_3 population, and therefore must have been homozygous for the maize character *Sd Sd*. Plants 9 and 8, however, gave a bimodal curve similar to the original F_2 population. These two plants must have been heterozygous, *Sd sd*. The F_3 populations from plants 10 and 2 are merely skewed to the left and are difficult to explain on the basis of a single genetic factor.

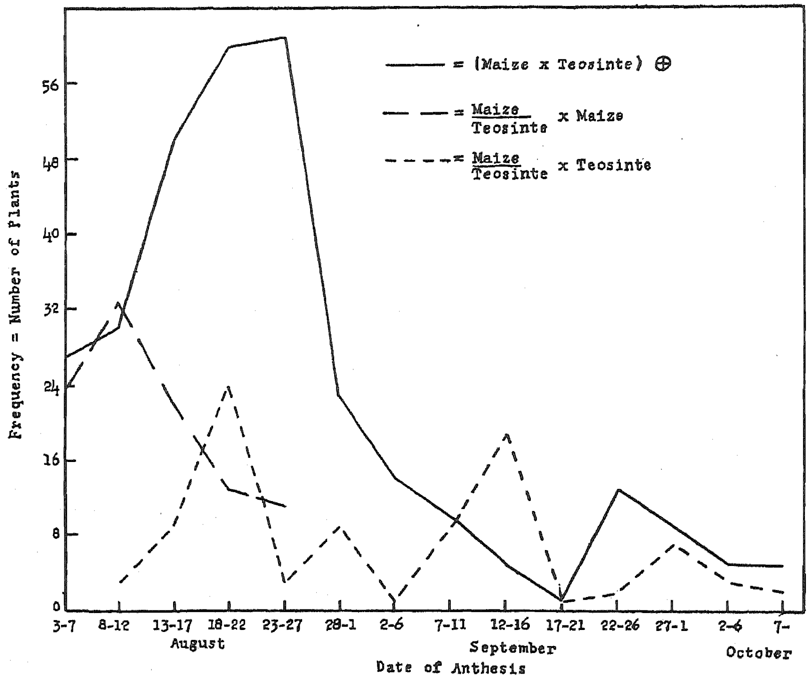

FIGURE 1.—The frequency distribution of the dates of anthesis of F_2 and backcross plants of a maize-teosinte hybrid (1937). The calendar from August 3rd to October 6th has been divided into 5-day intervals instead of daily intervals to make a smoother curve. This grouping has not destroyed any of the major modes.

In this discussion I have referred to the response of maize to length of day as the "maize character." Can it be said that maize is either a long-day or a short-day plant? Probably the best answer to that question is that maize is less sensitive to length of day than any truly long-day or short-day plant. We know that maize will flower in either the long days of the northern latitudes or the short days of the southern latitudes. We also know, however, that a strain of maize should not be moved more than 100 miles north or south from the region in which it is commonly grown. If moved south, it comes into reproduction before it has made suitable vegetative growth. If moved north, it produces good vegetative growth but

does not set seed early enough to escape killing frosts. In other words, for each latitude, strains of maize are selected which have the proper balance between vegetative growth and time of flowering for maximum production in that particular seasonal day length. These strains are referred to as having "medium" maturity. Other strains unadapted to a particular region are said to be either "early" or "late" strains. The early strains would

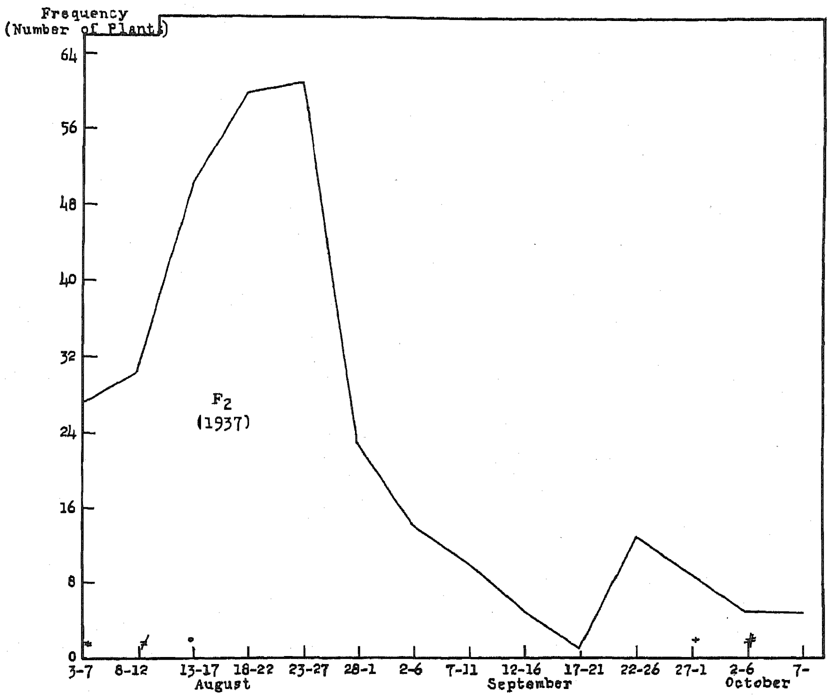

FIGURE 2.—The graph shows the frequency distribution of the dates of anthesis of 313 F_2 plants of a maize-teosinte hybrid in 1937. Seven of these F_2 plants (whose dates of anthesis are indicated) were selfed and their F_3 progeny grown in 1938. Table 1 shows the frequency distribution of the dates of anthesis of the F_3 plants. A killing frost on October 7th marked the completion of the experiment.

be more productive if moved farther north to longer days; whereas the late strains would be more productive if moved farther south to shorter day conditions. The above statements are made from observation of common cultural practices. In order to obtain more concrete evidence, however, an experiment was designed to study the effect of length of day on early, medium, and late strains of maize at Ithaca.

On May 19, 1938, a greenhouse bench 19 feet long by 5.5 feet wide was filled with soil and planted to ten seeds each of early, medium, and late-maturing maize, and two kinds of teosinte: Huixta (from Guatemala) and Durango (from Mexico). On June 9th when the seedling plants were

three to seven inches high, the bench was divided into five sections with black cloth. Each section included ten plants: two plants each of the five types listed above. (See figure 3.)

	6 hours		8 hours		10 hours		12 hours		14 hours	
Early maize	E	E	E	E	E	E	E	E	E	E
Medium maize	M	M	M	M	M	M	M	M	M	M
Late maize	L	L	L	L	L	L	L	L	L	L
Huixta teosinte	H	H	H	H	H	H	H	H	H	H
Durango teosinte	D	D	D	D	D	D	D	D	D	D

FIGURE 3.—Experimental design to test the effect of length of daily light period on time of flowering of maize and teosinte. The table represents a greenhouse bench 19 feet$\times 5\frac{1}{2}$ feet. Letters indicate the types and positions of the plants tested.

The black cloth was used to shade the sections a part of each day. The plants in section 1 were given only six hours of light daily. Sections 2, 3, 4, and 5 received 8, 10, 12, and 14 hours of light, respectively. This experiment was conducted throughout the growing season, from seedling stage to anthesis. The results of the test are given in table 2.

TABLE 2

Effect of length of day on time of flowering of maize and teosinte. The figures in the table are the days from seedling stage (three to seven inches) to first day of anthesis. The total number of days from date of planting to anthesis can be obtained by adding 21 days to each figure.

	6 HOURS	8 HOURS	10 HOURS	12 HOURS	14 HOURS
Early maize	37	38	36	36	39
Medium maize	43	43	43	43	50
Late maize	62	61	60	58	70
Huixta teosinte	101	101	112	120	—
Durango teosinte	42	42	41	40	74

By following each line of figures across from left to right we can see that there is no appreciable difference in time of flowering of plants exposed to 6, 8, 10, or 12 hours of light daily, except possibly in Huixta teosinte. But the increase in day length to 14 hours, delays the time of flowering of medium and late maize for periods of seven and ten days, respectively. Under the conditions of this experiment, then, maize shows some response to length of day. (Since only a small number of plants were tested, the results are interpreted as indicating direction of reaction, rather than degree of response.) Perhaps an increase in day length to 16 or 18 hours would have further delayed the time of flowering.

It will be noted from table 2 that the date of anthesis of Durango teosinte was delayed 34 days by increasing the day length from 12 to 14 hours. The Huixta teosinte (from Guatemala) flowered earliest in the shorter day lengths, and not at all in the 14-hour chamber. Guatemalan teosinte

would be expected to require a shorter day for reproduction than Mexican teosinte because of the shorter days of its natural habitat.

In this experiment Durango teosinte required 42 days of short-day treatment for reproduction, whereas Guatemalan teosinte required a minimum of 101 days. In a previous experiment (table 3) Durango teosinte flowered in about 35 days after short-day treatment was begun, and Guatemalan teosinte flowered in about 47 days.

TABLE 3

Effect of short-day treatment on teosinte strains.

TEOSINTE	DATE OF PLANTING	NUMBER OF PLANTS	SHORT-DAY TREATMENT BEGUN	SILKS	POLLEN	DAYS TO SILKS
Mexican—						
Durango	May 30	4	July 13	Aug. 16	Aug. 19	35
Nobogame	May 30	8	July 13	Aug. 12	Aug. 16	31
Novocayan	May 30	3	July 13	Aug. 15	Aug. 19	34
Guatemalan—						
Huixta	May 30	13	July 13	Aug. 28	Sept. 1	47

The essential difference between these two experiments is that in the first case, short-day treatment was begun when the plants were in the seedling stage; whereas in the second case, the plants were about six weeks old before being submitted to shorter day conditions. The necessity for longer treatment of seedlings might be explained by assuming that a teosinte plant must attain a certain stage of development before short-day treatment is effective in stimulating reproduction.

One problem which complicates the study of photo-periodism in maize-teosinte hybrids is the separation of earliness and lateness factors from response to length of day. If an F_2 plant from a maize-teosinte hybrid flowers in the latter part of the season, does it flower late because of lateness factors or because it needs the shorter days of the late summer to stimulate reproduction?

This question cannot be answered satisfactorily from any of the data obtained by the writer. One method of approach to the question, however, was to study the relative dates of anthesis of maize, teosinte, and their F_1 hybrid under long-day conditions. The results of this experiment are shown in figure 4.

The F_1 hybrid flowered somewhat later than the parental maize, and much earlier than the parental teosinte. Is this F_1 distribution due to partial dominance of the maize character, or to lateness factors from teosinte? If we could be sure that teosinte contributed lateness factors to the hybrid, then the delayed F_1 curve would be attributed to those factors.

On the other hand, if we could be sure that the flowering date of teosinte, irrespective of response to length of day, were similar to that of the parental maize, which is of medium maturity, then it could be said that the weak response to length of day in maize is not completely dominant over the strong response to length of day in teosinte.

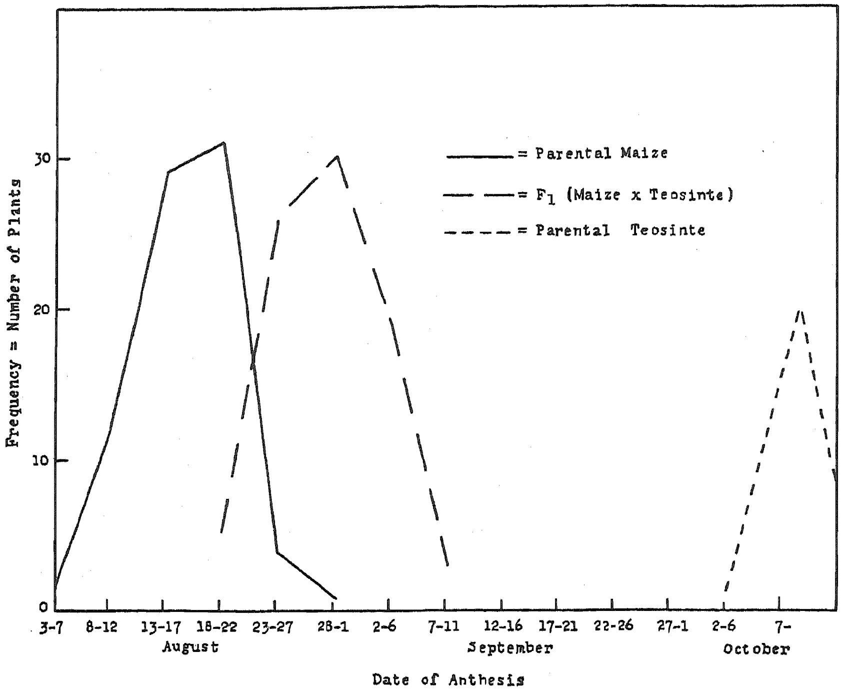

FIGURE 4.—A comparison of the frequency distributions of the dates of anthesis of maize, teosinte, and their F_1 hybrid.

Further information could be obtained by growing maize, teosinte, and their F_1 hybrid in the greenhouse in winter under short-day conditions. This test was not satisfactorily completed because the light in winter was so weak that it had to be supplemented with mazda lights. Artificial illumination was given the plants from 4 P.M. to 10 P.M. each day. The dates of anthesis were recorded and are given in table 4.

The F_1 hybrid flowered earlier than either the maize parent or the teo-

TABLE 4

	DATE OF PLANTING	DAYS TO POLLEN
Parental maize	Dec. 20, 1936	122 (mean of 7 plants)
Parental teosinte	Dec. 20, 1936	176 (mean of 2 plants)
F_1 hybrid	Dec. 20, 1936	100 (mean of 8 plants)

sinte parent. The maize was highly inbred and relatively weak. Perhaps the more rapid development of the F_1 hybrid was due to its advantage in vigor.

LINKAGE RELATIONS OF *sd*

Since the response to short day in teosinte is controlled by a simple Mendelian factor, *sd sd*, and since crossing over between the teosinte chromosomes and their maize homologues is normal (EMERSON and BEADLE 1932), the linkage relations of *sd sd* with qualitative maize characters should show the locus of the *sd sd* gene.

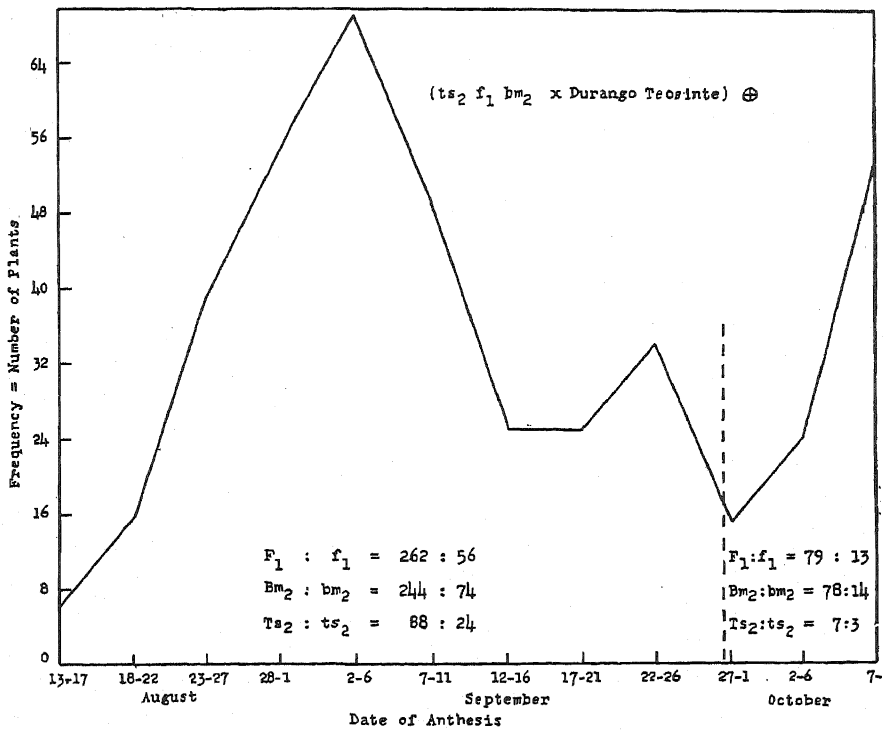

FIGURE 5.—Test for linkage of *sd sd* with genes in chromosome 1.

For this study, teosinte was crossed with tester stocks of maize for chromosomes 1–6, inclusive, in the summer of 1937. The F_1 plants were grown in the greenhouse during the winter of 1937–38 and F_2 seed was obtained. The seed did not mature until late spring, however, and could not be planted until June 3. That was about 18 days later than the most desirable time of planting. This delay was accentuated by two weeks of drouth, and the plants did not appear above ground until the latter part of June. This late start of the seedlings caused the first mode of the dates of anthesis to partially overlap the second mode. The date of the second

mode apparently was not substantially changed because, as mentioned previously, the 13-hour day begins about September first, and 30 days of short-day treatment are sufficient to bring teosinte (or in this case, an F_2 segregate) into reproduction. Actually, then, the second or minor mode was held constant, while the first or major mode was advanced toward it about one month. (See figures 5 to 10.)

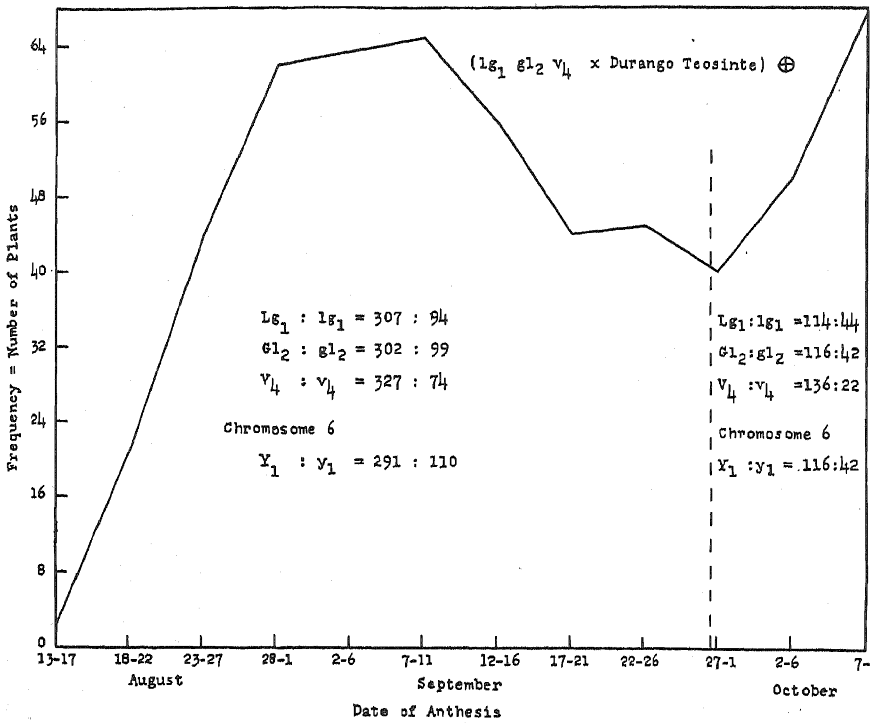

FIGURE 6.—Test for linkage of *sd sd* with genes in chromosome 2.

The method of testing for linkage between the gene *sd sd* (short day) and qualitative maize genes whose loci are known, was to study the segregation of the known genes in the major mode and the minor mode of the F_2 distribution. Assuming that the minor mode consists of homozygous *sd sd* plants, while the major mode is made up of both *Sd Sd* and *Sd sd* plants, independent inheritance of *sd sd* with respect to a known maize gene, *A-a*, would be shown by a 3:1 ratio of *A-a* in each mode.

The data in figures 5–10 show that *sd sd* is not linked with any of the marker genes in chromosomes 1, 2, 3, 4, 5, and 6 used in this study.

DISCUSSION

This study of photoperiodism in Zea-Euchlaena hybrids is genetical, not physiological.

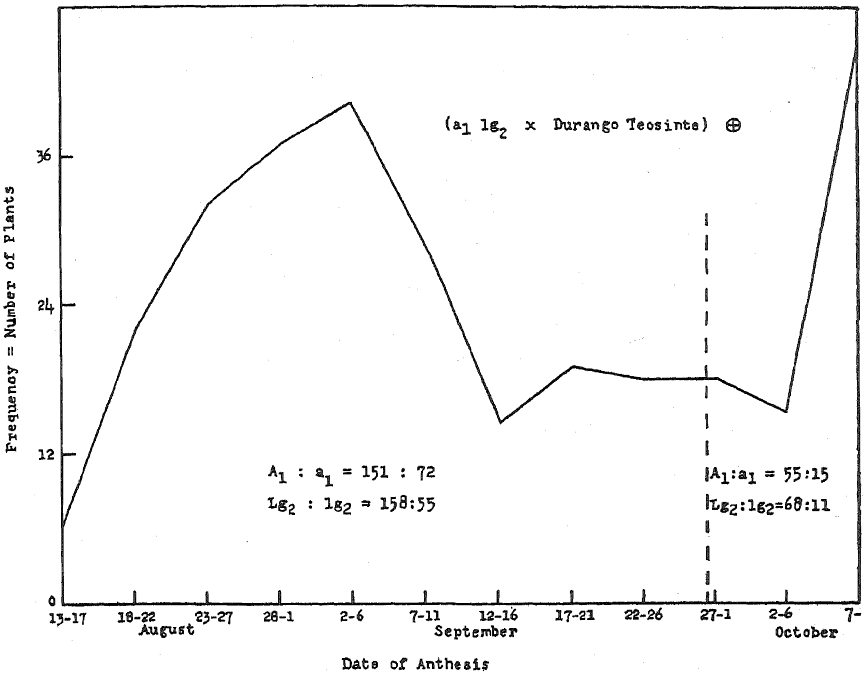

FIGURE 7.—Test for linkage of *sd sd* with genes in chromosome 3.

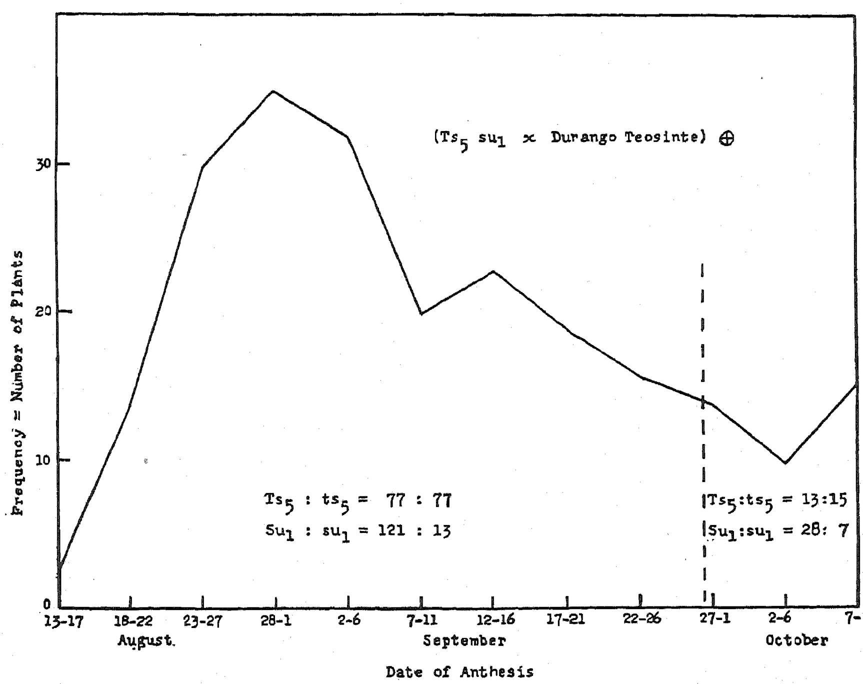

FIGURE 8.—Test for linkage of *sd sd* with genes in chromosome 4.

Perhaps the most convincing evidence of the simplicity of the inheritance of the response to short day is shown by the data in figures 1 and 2. The distribution of the dates of anthesis in F_2 and reciprocal backcross populations follows a unifactorial pattern. This evidence of Mendelian inheritance is enhanced by the breeding behavior of F_2 plants taken at different points in the bimodal distribution. F_2 plants from the second mode gave, on selfing, F_3 populations whose dates of anthesis formed a

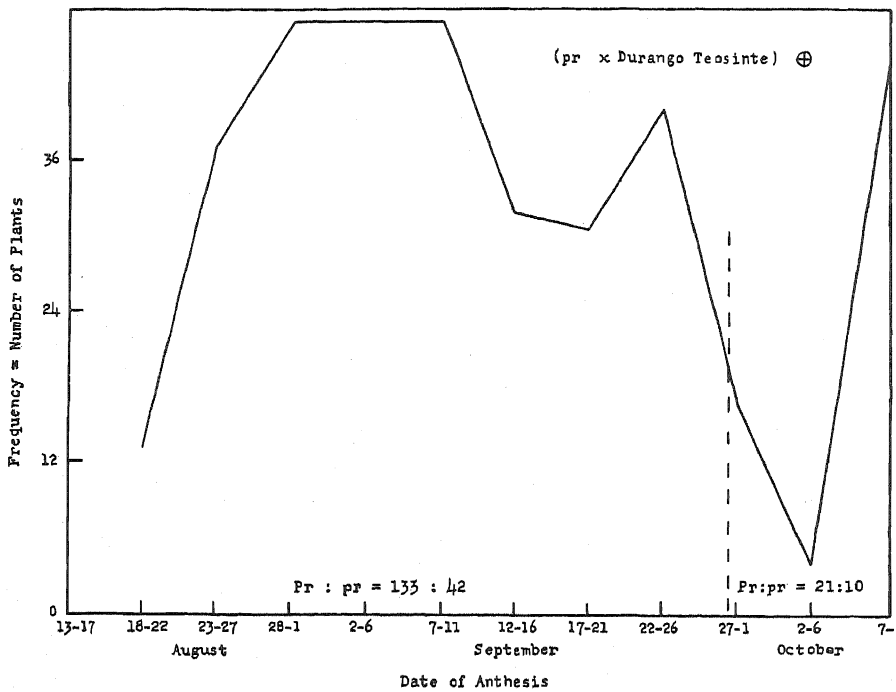

FIGURE 9.—Test for linkage of *sd sd* with genes in chromosome 5.

similar late mode; while F_2 plants from the first mode gave, on selfing, F_3 populations whose dates of anthesis simulated the original bimodal F_2 distribution. The F_3 population from one F_2 plant in the first mode, gave a unimodal curve similar to the first mode, indicating that the selfed F_2 plant was homozygous *Sd Sd*.

If, in maize, the normal allele, *Sd Sd*, of the recessive teosinte response to short day, *sd sd*, mutated to the teosinte form, late-maturing segregates would occur in the ordinary field of corn. Evidence that this or a similar mutation does occur has been presented by three authors: (1) BRUNSON (1935) who reported a plant which gave indefinite vegetative growth under field conditions, with no evidence of either tassel or ear shoot. It could be brought into flowering under short-day conditions. (2) BRYAN (Maize

Cooperation News Letter, March 1938) who described a tall, late-maturing type of plant, which when crossed with medium-maturing types, gave normal F_1 plants; and (3) EMERSON (unpublished), who has informed the writer of a similar late-maturing type, which under Ithaca conditions, will segregate as a simple recessive.

BRUNSON'S late-flowering type, *cz*, has been lost due to inviability of old seed stocks. F_2 seed of the types reported by BRYAN and by EMERSON was

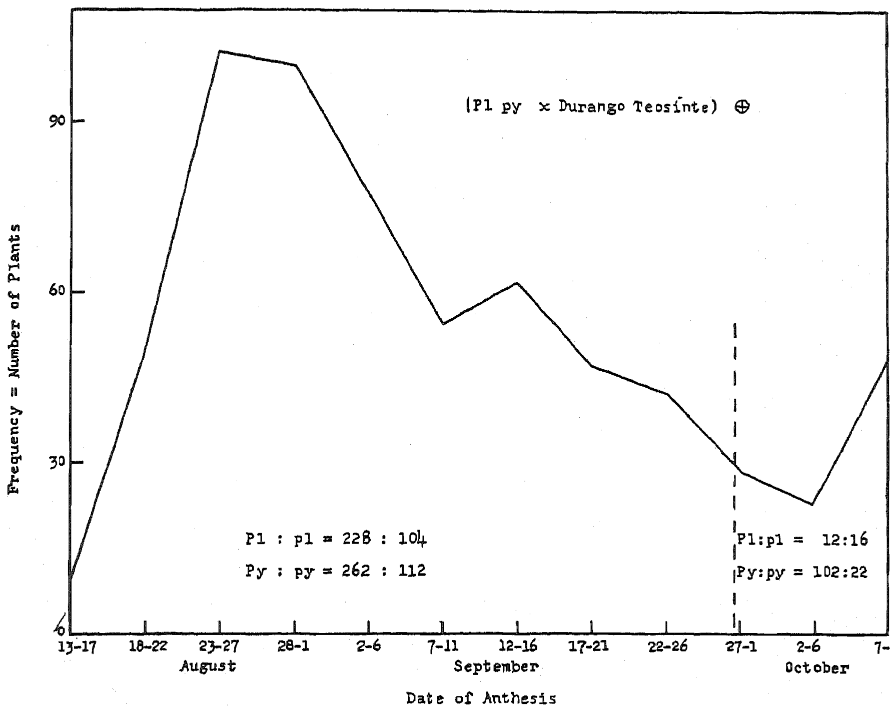

FIGURE 10.—Test for linkage of *sd sd* with genes in chromosome 6.

grown in Ithaca in 1938 and late-flowering plants segregated in a Mendelian manner. One of the segregates from Bryan's stock was crossed with Durango teosinte to test for allelism with the teosinte response to short day. The test will be made in 1939.

Summarizing this section we may say that the response to short day in teosinte is controlled by a simple Mendelian factor, and that mutations to short-day plants occur in ordinary maize.

INHERITANCE OF SPIKELET DIFFERENCES

Paired versus single female spikelets

COLLINS and KEMPTON (1920) reported that of the 33 characters studied in an F_2 population from a cross between Tom Thumb pop corn and Flor-

ida teosinte, the nearest approach to Mendelian inheritance was shown by the arrangement of the spikelets in the pistillate inflorescence. In maize the female spikelets are borne in pairs; in teosinte the female spikelets are borne singly. Dominance of the maize character was complete in the first generation. In the second generation the segregation was not complete; many plants had both single and paired female spikelets, but the number of individuals in which paired spikelets predominated was approximately three times (34 paired to 12 single) the number in which there were more single female spikelets.

Data obtained by the writer from crosses between maize and Durango teosinte agree with the findings of COLLINS and KEMPTON that paired versus single spikelets is a unifactorial difference, but disagree in showing that dominance of paired spikelets is not complete in the F_1 hybrid. Most of the F_1 ears have paired spikelets, but some of them have single spikelets.

TABLE 5

Segregation of paired and single spikelets in F_2 and reciprocal backcross populations from maize-teosinte hybrids.

	F_2		$F_1 \times$ MAIZE		$F_1 \times$ TEOSINTE	
	OBS.	CALC. (3:1)	OBS.	CALC. (1:0)	OBS.	CALC. (1:1)
Paired spikelets	921	904.5	142	142	23	21
Single spikelets	285	301.5	0	0	19	21

In the F_2 population three types of plants are found: those with all single spikelets, those with all paired spikelets, and those with both paired and single spikelets. The ratio of these three types is approximately 1:1:2. In the backcross to maize, all the ears have paired spikelets. In the backcross to teosinte, approximately one-half of the plants have all single spikelets, and one-half of them have both paired and single spikelets.

If, then, plants with both paired and single spikelets are grouped in the class with those plants having all paired spikelets, simple ratios are obtained (table 5).

The data in the above table confirm the hypothesis of unifactorial inheritance of paired versus single spikelets as described by COLLINS and KEMPTON.

The recessive single spikelet condition of teosinte is given the symbol, *pd pd*, and its allele in maize is *Pd Pd*.

The data in table 6 show that an F_2 plant with single spikelets will breed true for single spikelets in the F_3 generation; whereas an F_2 plant with paired spikelets may either breed true for paired spikelets in the F_3 or segregate for single spikelets.

TABLE 6

Segregation of paired and single spikelets in F_3 populations from maize-teosinte hybrids.

F_2 PLANT NUMBER	DESCRIPTION OF F_2 PLANT	NO. OF F_3 SEGREGATES PAIRED	SINGLE	PROBABLE GENOTYPE OF F_2 PLANT
1	Paired Spikelets	38	0	*Pd Pd*
8	Single Spikelets	0	55	*pd pd*
9	Single Spikelets	0	15	*pd pd*
33	Single Spikelets	0	81	*pd pd*
2	Paired Spikelets	42	6	*Pd pd*
6	Paired Spikelets	10	1	*Pd pd*
10	Paired Spikelets	10	10	*Pd pd*
12	Paired Spikelets	24	7	*Pd pd*

Single spikelets, *pd pd*, shows independent inheritance with the marker genes for chromosomes 1, 2, 3, 4, 5, and 6 used in this test. *Lg2* and *pd* showed only 31 percent recombination with a P value of 0.015 and should be tested further (table 7).

Since the difference between the paired and single spikelet condition is controlled by a single genetic factor, a mutation from paired to single spikelets might be expected to occur in ordinary maize. The chances that this mutation would be noticed in the resulting plants are not very great, however, especially in a culture with high row numbers. The change would

TABLE 7

*Linkage relations of single spikelets, **pd pd**, with known qualitative maize genes.*

CHROM.	GENES	LINKAGE PHASE	NUMBER OF INDIVIDUALS *XY*	*Xy*	*xY*	*xy*	TOTAL	PERCENT RECOMB.
1	*F1 Pd*	R	144	39	37	5	225	40
	Bm2 Pd	R	140	34	41	10	225	50
2	*Lg1 Pd*	R	160	38	50	9	257	46
	Gl1 Pd	R	154	34	58	11	257	48
	B Pd	C	40	28	36	11	115	60
	V4 Pd	R	167	38	43	9	257	49
3	*A1 Pd*	R	94	19	42	5	160	43
	Lg2 Pd	R	100	22	36	2	160	31
4	*Ts5 Pd*	C	50	15	18	10	93	41
	Su1 Pd	R	46	26	7	4	83	50
5	*Pr Pd*	R	54	20	20	7	101	49
6	*Y1 Pd*	C	155	35	55	12	257	49
	Pl Pd	C	73	25	34	13	145	48
	Py Pd	R	166	87	37	10	300	41

simply reduce the row number by one-half, and a 16-row type would show a few 8-row ears which would probably be ignored.

A mutation to single spikelets in an inbred line of maize was discovered by the writer when he crossed maize with teosinte. The F_1 ears had single spikelets instead of the usual paired spikelets. The F_2 ears, likewise, were homozygous for single spikelets. The inbred line of maize used in this cross has a fasciated type of ear, and its single spikelets would not have been noticed except for the cross with teosinte.

Summarizing this section, it has been shown that the paired spikelet condition of maize is dominant over the single spikelet condition of teosinte, and is a simple Mendelian character; and that mutation from paired to single spikelets occurs in maize.

INHERITANCE OF TASSEL AND EAR DIFFERENCES

Two-ranked versus many-ranked central branch of the tassel

The two-ranked central branch of the teosinte tassel[2] as contrasted with the many-ranked central branch (or central spike) of the maize tassel presents one of the characteristic differences between maize and teosinte. How is this character inherited in hybrids between the two genera?

The F_1 plant has a many-ranked central branch in its tassel, similar to

TABLE 8

The segregation of the two-ranked teosinte type of central branch in F_3 populations from maize-teosinte hybrids.

F_2 PLANT NUMBER	DESCRIPTION OF F_2 PLANT	NUMBER OF F_3 SEGREGATES MANY-RANKED	TWO-RANKED	PROBABLE GENOTYPE OF F_2 PLANT
9	Two-ranked	0	16	*tr tr*
1	Many-ranked	58	0	*Tr Tr*
8	Many-ranked	59	0	*Tr Tr*
2	Many-ranked	26	13	*Tr tr*
6	Many-ranked	5	6	*Tr tr*
10	Many-ranked	15	3	*Tr tr*
12	Many-ranked	32	18	*Tr tr*

the maize parent. In the F_2 population, the two-ranked teosinte type of central branch segregated sharply as a simple Mendelian character (figure 11). The F_2 population consisted of 890 plants with many-ranked and 316 plants with two-ranked central spikes.

The recessive two-ranked central branch of the tassel is given the symbol, *tr tr*, and its dominant allele, the many-ranked central branch of the maize tassel, is designated by *Tr Tr*.

[2] Contrary to general belief, the central branch of the teosinte tassel is a continuation of the axis of the panicle or rachis, and not a branch from the rachis.

The data in table 8 show that an F_2 plant with a two-ranked central branch will breed true for the two-ranked condition in the F_3 (according to the one F_2 plant tested); whereas an F_2 plant with a many-ranked central branch may either breed true for the many-ranked condition, or segregate for the two-ranked condition.

This study of the teosinte type of tassel as contrasted with the maize

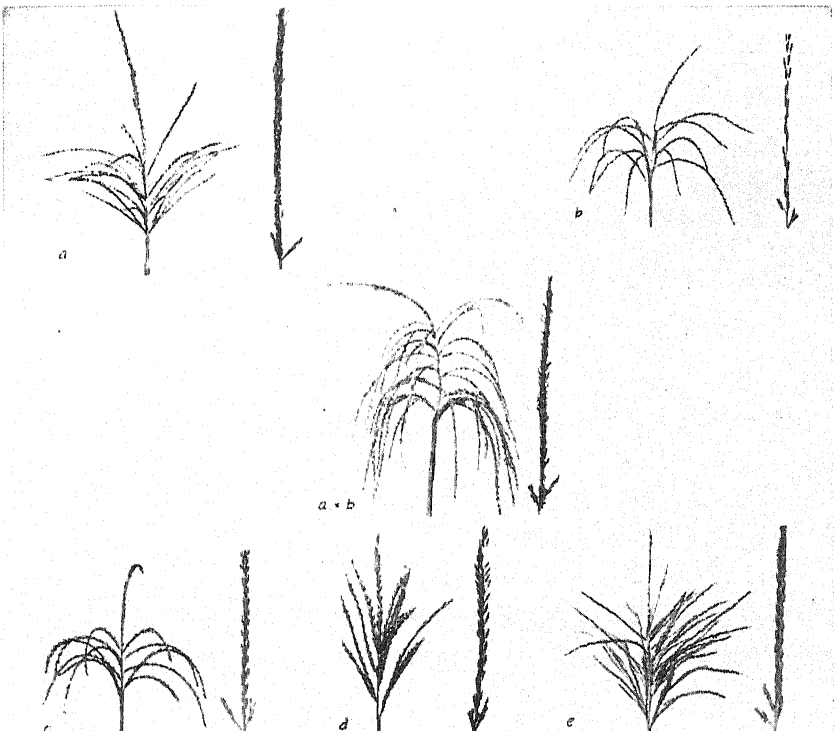

FIGURE 11.—The inheritance of many-ranked (*Tr Tr*) vs. two-ranked (*tr tr*) central branch of the tassel in maize-teosinte hybrids. Inset: enlargement of central branch.
a—parental maize (*Tr Tr*).
b—parental teosinte (*tr tr*).
a×b—F_1 hybrid (*Tr tr*).
c, d, e—Teosinte-like segregates (*tr tr*) in F_2.

type includes only the difference between a two-ranked central branch and a many-ranked central branch. It does not pretend to be a complete study of tassel type. Other factors to be considered in a more detailed study would be total number of spikelets; size of spikelets; number, length, and positions of lateral branches; articulation; and others. The wide range of tassel types in maize is a continuous series which suggests multiple factor inheritance.

The distinction between a two-ranked and a many-ranked central

branch, on the other hand, is discontinuous, and the two types can be readily classified in segregating populations.

Two-ranked versus many-ranked ear type

As will be seen from this study, the two-ranked central branch of the teosinte tassel and the two-ranked ear of the teosinte plant are directly related and are both controlled by the same recessive gene, *tr tr*. Moreover, the many-ranked central branch (or central spike) of the maize tassel and the many-ranked ear of the maize plant are directly related and are both controlled by the same dominant gene, *Tr Tr*.

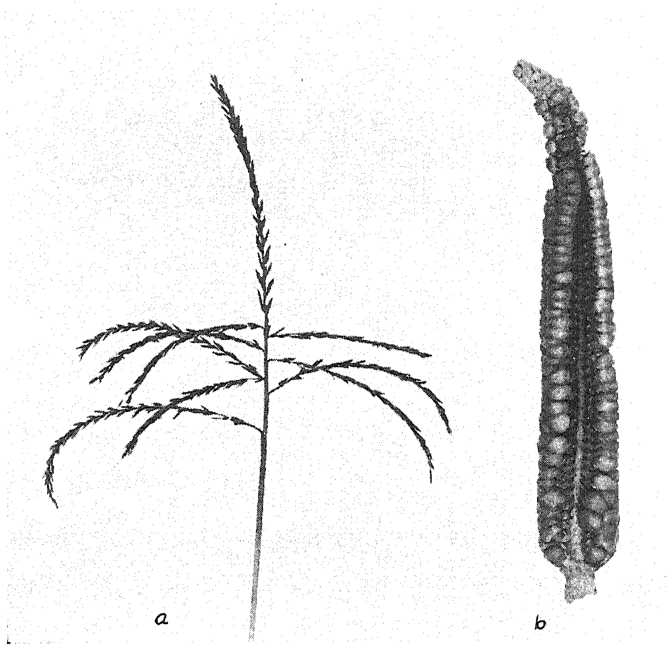

FIGURE 12.—Mutant form of maize with four-rowed (two-ranked) ear and two-ranked central branch of the tassel.

In maize-teosinte hybrids most of the F_1 ears are two-ranked, but a few of them are many-ranked. These two-ranked ears occur laterally on the side branches in the axils of the leaves, while the few many-ranked ears occur terminally on the side branches. (Most of the side branches are terminated by staminate inflorescences, not pistillate inflorescences.) In spite of the predominance of two-ranked ears in the F_1 hybrid, the many-ranked condition of maize is considered dominant over the two-ranked condition of teosinte, because (1) the central branch of the F_1 tassel is many-ranked; (2) in segregating populations, two-ranked ear is associated with two-ranked central branch and many-ranked ear with many-ranked

central branch; and (3) four-rowed (two-ranked) mutant form of maize (see figure 12) has a two-ranked central branch in its tassel: when this mutant form is crossed with ordinary maize (TAVCAR 1935), the F_1 plant has a many-ranked ear and a many-ranked central branch. In the F_2 population the mutant form segregates as a simple Mendelian character. Furthermore, this two-ranked mutant form of maize is allelic with the two-ranked condition of teosinte, as shown by test crosses with teosinte.

TABLE 9

Linkage relations of two-ranked, tr tr, condition with known qualitative maize genes.

CHROM.	GENES	LINKAGE PHASE	NUMBER OF INDIVIDUALS					PERCENT RECOMB.
			XY	*Xy*	*xY*	*xy*	TOTAL	
1	*F1 Tr*	R	140	44	32	9	225	48
	Bm2 Tr	R	127	46	45	7	225	38
2	*Lg1 Tr*	R	144	53	49	11	257	43
	Gl1 Tr	R	138	50	55	14	257	45
	B Tr	C	57	11	38	9	115	47
	V4 Tr	R	156	53	37	11	257	48
3	*A1 Tr*	R	87	26	40	7	160	42
	Lg2 Tr	R	93	29	32	6	160	43
4	*Ts5 Tr*	C	38	4	41	10	93	38
	Su1 Tr	R	61	11	8	3	83	60
5	*Pr Tr*	R	44	30	19	8	101	43
6	*Y1 Tr*	C	145	45	48	19	257	47
	Pl Tr	C	61	37	31	16	145	52
	Py Tr	R	183	80	34	3	300	28

In classifying ear type in an F_2 population from a maize-teosinte hybrid, then, the type of central branch in the tassel serves just as well as the ears themselves. A plant homozygous for the gene, *tr tr*, has a two-ranked ear and a two-ranked central branch. A plant homozygous *Tr Tr* or heterozygous *Tr tr* has a many-ranked ear and a many-ranked central branch.

The F_2 Mendelian ratio and the F_3 ratios in table 8 apply not only to the inheritance of tassel type (two-ranked or many-ranked), but also to the inheritance of ear type.

Two-ranked, *tr tr*, shows independent inheritance with the marker genes used from chromosomes 1, 2, 3, 4, and 5; and possible linkage with pigmy, *py py*, in chromosome 6. Due to the fasciation of the pigmy tassel, this linkage is subject to further test with other genes in that region of chromosome 6.

These data show that *tr tr* and *pd pd* are on the same chromosome, and are 20 crossover units apart.

The number of the maize chromosome which carries the genes *Pd Pd* and *Tr Tr* is not known. The data in table 9 indicate that *Tr Tr* is linked with the gene, *py py*, on chromosome 6. The writer has little confidence in that linkage, however, because of the fasciation of the pigmy tassel.

The 20 percent recombination of *Pd pd* and *Tr tr* makes possible the production of ears with an odd number of kernel rows, that is, F_2 plants with the genetic constitution *pd pd Tr Tr* or *pd pd Tr tr* (single female spikelets, many-ranked) may have any number of rows from 2 to 13, inclusive. Ears with an odd number of rows have been found.

TABLE 10

Linkage relations of two-ranked, tr tr, with single spikelets, pd pd.

GENES	LINKAGE PHASE	NUMBER OF INDIVIDUALS					PERCENT RECOMB.
		XY	Xy	xY	xy	TOTAL	
Tr Pd	C	795	95	126	190	1206	20

In summary of this section, it may be said that the two-ranked ear and the two-ranked central branch of the teosinte tassel are both controlled by the same recessive gene, *tr tr;* and that in maize, the dominant gene, *Tr Tr* (many-ranked) mutates to the recessive form, giving a plant with a two-ranked ear and a two-ranked central branch.

GENERAL DISCUSSION

One of the theories of the origin of maize is that it arose from teosinte by a relatively few large-scale mutations. This theory, however, is not widely accepted. One of the most serious objections raised against it is that the characters which differentiate the two genera are numerous and show multiple factor inheritance, which suggests that maize and teosinte differ by hundreds if not thousands of genes.

Actually, however, when all the characters which show wide variation in both maize and teosinte are omitted, and only the characteristic differences are considered, the list of differences is reduced to about five as follows:

Weak versus strong response to length of day
Paired versus single female spikelets
Many-ranked versus two-ranked ear (and central branch of the tassel)
Membranous versus horny glume
Naked versus covered seed.

Width of leaves, tillering, sturdiness of the plant, and similar characters,

cannot be considered characteristic differences because each shows extreme variation in both maize and teosinte.

As shown by the experimental results outlined in this paper, at least three of the characteristic differences are inherited as simple Mendelian characters. This unifactorial inheritance tends to obviate the speculative suggestion that maize and teosinte differ by hundreds if not thousands of genes, and makes the theory of the origin of maize from teosinte by mutation a plausible one.

Perhaps if a number of inbred lines of teosinte were maintained over a period of years, mutations to maize-like characters would occur and could be combined to synthesize maize. This possibility does not seem fantastic in view of the fact that mutations to teosinte-like characters have been found in inbred lines of maize (paired to single female spikelets, many-ranked to two-ranked condition, and weak to strong response to length of day).

SUMMARY

1. The weak response to length of day in maize is dominant over the strong response to length of day in teosinte, and segregates as a simple Mendelian character. Mutation to the teosinte form occurs in maize.

2. The character paired female spikelets of maize is dominant over single female spikelets of teosinte, and shows simple inheritance. Mutation from paired to single spikelets occurs in maize.

3. The many-ranked ear of maize is dominant over the two-ranked ear of teosinte and segregates as a unifactorial difference. Two-ranked ear is associated with two-ranked central branch of the tassel, and many-ranked ear with many-ranked central branch. Mutation to the teosinte form (two-ranked) occurs in maize.

4. The gene, *pd pd* (single spikelets), is linked with the gene, *tr tr* (two-ranked), with 20 percent recombination.

5. The results are interpreted as supporting the theory of the origin of maize from teosinte by a relatively few large-scale mutations.

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ABSTRACTS OF PAPERS PRESENTED AT THE 1939 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

COLUMBUS, OHIO, DECEMBER 28–30, 1939

E. W. LINDSTROM, *Secretary*
Department of Genetics
Iowa State College, Ames, Iowa

ATWOOD, SANFORD S., U. S. Regional Pasture Research Laboratory, State College, Pa.: *Cytogenetics of incompatibility in Trifolium repens.*——Thirteen self-sterile F_1 plants were crossed in all combinations and were backcrossed with their self-sterile parents. The F_1 plants consisted of four intra-sterile, inter-fertile groups of five, four, three, and one plant, respectively, and all were reciprocally fertile with both parents. Using ten flowers in each cross, compatible crosses averaged 44.9 seeds and incompatible 0.26. A second series of diallel crosses were made between $13F_1$ plants and their parents. One parent was self-sterile and the other highly "pseudo-self-fertile." Both this latter parent and its progeny were practically self-sterile when individual flowers were self-pollinated in either full flower or the bud, but they set selfed seed when the entire heads were rubbed to effect pollination. The factors conditioning this pseudo-self-fertility are independent of those causing cross-incompatibilities, since four intra-sterile, inter-fertile groups of four, four, three, and two plants, respectively were found in this second set of progeny. Compatible crosses averaged 41.9 seeds and incompatible 0.18. The results in both series of crosses are explained best by the diploid (16 bivalents found regularly) personate type of oppositional factors where the parents differ in both alleles. The 12 groups from the two series were compatible in all combinations, indicating at least seven different allelomorphs in the four parents. Other evidence suggesting a large number of allelomorphs is that all matings (over 200) between unrelated plants were compatible. Few or no seeds were set in incompatible crosses because of poor pollen germination and pollen tube growth within a few hours after pollination.

BANTA, S. M., Brown University, Providence, R. I.: *Possible rôle of amixis in the evolution of organisms.*——Experimental findings from parthenogenetic reproduction in Cladocera and other well authenticated facts suggest that amixis, particularly when alternating with amphimixis, may be a potent factor in evolution. In Daphnia, mutation in diploid parthenogenesis is moderately frequent——all of our recognized Cladocera mutations having arisen during parthenogenesis. Most of these mutations are recessives and their effects, almost exclusively physiological, are recognized only in the results of sexual reproduction——inbreeding within the clone involved. The relative frequency of occurrence of these mutations during parthenogenesis together with, in nature, the periodical occurrence of amphimixis suggests that in the amictic-amphimictic cycle abundant genic recombinations occur.

The vast populations normally resulting from amixis should contain proportional numbers carrying any recessive mutations, or any non-deleterious dominant mutations, which might have arisen. If the mutation is dominant and confers an advantage to its possessor the mutant population during parthenogenesis should become disproportionately large. In any case, if the mutation is of potential advantage the amictic-amphimictic cycle affords opportunity for the one-in-a-million recombination which, perhaps alone, may produce an individual superior to its forbears or fitted for occupancy of a changed or new environment. For example, our thermal mutation in *Daphnia longispina* was such a potentially advantageous mutation. The amictic-amphimictic cycle occurs in many organisms, particularly among parasites and animals occupying transitorally favorable environments. It is conceivable that this mechanism has been a large factor in the development of the almost incredibly specialized and elaborate adaptations in life histories of certain parasites.

BARTO, ELIZABETH, Laboratory of Vertebrate Genetics, University of Michigan, Ann Arbor, Mich.: *Absence of linkage between certain characters in the deer-mouse, Peromyscus maniculatus.*——The characters albino versus dilute, albino versus nude, and ivory versus hairless, in the deer-mouse, *Peromyscus maniculatus*, have been tested to determine their linkage relationships. Independent assortment of the genes for each of these pairs of characters was shown in the F_2 generations and in test-crosses, indicating absence of linkage.

BEATTY, ALVIN V. University of Alabama, University, Ala.: *Mitosis in the pollen tube of Eschscholtzia.*——Within fifteen minutes after sowing the pollen of the California poppy on a prepared medium, both the growth of pollen tubes and the division of generative nuclei have begun. All normal mitotic prophase stages, including the double nature of the chromosomes, can be seen. At the end of two hours the pollen tubes range in length up to 2 mm. Most tubes between 0.5 mm and 1.0 mm show a typical mitotic metaphase with a well developed spindle and an equatorial alignment of the chromosomes. Since the chromosomes are small they are situated throughout their lengths in the equatorial plane. The anaphase chromosomes are separate, easily distinguishable, and migrate simultaneously, forming regular mitotic configurations. Immediately following telophase the gametes are formed. They migrate toward the end of the tube, but are rarely closer together than the distance between the telophase nuclei.

BLAIR, ALBERT P., Indiana University, Bloomington, Ind.: *Inter-relations of the toads of eastern North America.*——Inter-relations of the toads of eastern North America have been studied from three angles: (1) extensive field work involving determination of ranges, areas of hybridization, etc., (2) critical

examination of specimens collected in the field, and (3) experimental hybridization. The several so-called species are found in almost every case to be linked by intermediate populations. 3500 toads have been measured and local variation studies made where sufficient specimens are available. 987 species hybrids representing 29 crosses have been raised through metamorphosis.

BLAKESLEE, A. F., AVERY, A. G., and BERGNER, A. D., Carnegie Institution of Washington, Cold Spring Harbor, New York: *Genes associated with prime types in Datura and their possible relation to the hypothesis of position effect.*——In our collection of over ninety prime types which consists chiefly of races homozygous for segmentally interchanged chromosomes, the large majority show no deviation from normal in appearance as would be expected if position effect were frequent. In a few cases, however, homozygous prime types are distinctly abnormal. It has not yet been possible to determine whether these exceptions are due to genes which do not cross over, to small deficiencies which accompanied the break, or to position effect.

BLANC, RICHARD, and BRAUN, WERNER, Department Zoology, University of California, Berkeley, Calif.: *Phenocopies and X-radiation in Drosophila melanogaster.*——The authors have attempted to establish a relationship between number of phenocopies in *Drosophila melanogaster* and extent of X-radiation. Prepupae of an inbred Oregon-R stock were subjected to X-radiation for periods of $\frac{1}{4}$ to $4\frac{1}{2}$ minutes (at approximately 940 r units per minute), and the images were examined for phenocopic effects. Characters most frequently noted were abnormal abdomen, various bristle defects (particularly absence), curled and curved wings, opaque wings, and trident. Occasional phenotypes included rough eyes, vortex, and a number of wing shape and wing venation effects. A direct proportionality between average number of phenocopies per fly and degree of radiation was observed from 1410 r units to the highest dosage for which significant data were available, i.e., 3290 r units. The decrease in number of normal flies is represented by an S curve. A typical mortality curve was found, with a sharp drop in viability from 2350 to 3290 r units. Certain relationships were established among the thoracic bristles. The anterior and posterior dorsocentrals reacted differently to increase in radiation, while the anterior and posterior scutellars reacted in a similar manner, though not to the same degree. It is suggested that the scutellars may be classified as a single bristle system, and the anterior and posterior dorsocentrals as separate bristle systems.

BRAUN, WERNER, Department of Zoology, University of California, Berkeley: *Increase in time of development after partial and complete starvation of larvae and its effect on the phenotype of several mutants of D. melanogaster*——Partial (peptone-food) and complete starvation of larvae of *D. melanogaster* increases time of development. Developmental rate can be changed differently accord-

ing to the degree and length of starvation and the stage in development at which it is started. If the time of development is thus increased in vg^{no} larvae an increasing destruction of wing area can be observed with increasing time of development (Proc. Nat. Acad. Sci. **25**, 5). Precisely timed tests indicated that no definite sensitive period exists for the effect of partial starvation. The effect is larger the earlier the larvae are transferred to peptone food. After partial starvation the destruction always increases from the tip of the wing towards the base of the wing (scalloping).——No definite sensitive period for complete starvation has been found as yet. *Patterns* of destruction are typically different if starvation is started severally at different times of larval development. Larvae which are completely starved for a 3 day period, which starts before the larvae are 75 hours old, develop into flies 80% of which always exhibit a destruction starting from the posterior margin of the wing (*Bd* type). If larvae are completely starved beginning at a time after 75 hours of larval age, all hatching flies exhibit wing destruction starting from the anterior margin (*Bx* type). An antler-like pattern of destruction results (60% of hatching flies) if larvae were removed from all food at the age of 50 hours. Changes of developmental rates were produced by the same method in some other mutants of *D. melanogaster*. It was observed that after increase in time of development *B*/+ and Lobe eyes become normal, Curly wings straighten out (only in certain stocks) and the amount of extra venation is reduced in plexus stocks.

BREHME, KATHERINE S., Carnegie Institution, Cold Spring Harbor, N. Y.: *The growth of transplanted Minute and wild type optic disks in Drosophila melanogaster.*——As part of a study of the effect of the host upon growth of imaginal tissue, optic disks of female larvae 76 hours after hatching were transplanted into larvae of the same age and sex (at 25°C). At eclosion of the host, the transplants were dissected out and the facets counted. The stocks used were Florida wild type (puparium formation at 100 hours after hatching) and Minute-w isogenic with Florida (puparium formation at 144 hours). The mean facet number of *Mw* transplants in wild type hosts, pupating 24 hours after the operation, was 294.3 ± 31.8; *Mw* transplants *Mw* hosts, pupating 68 hours after the operation, had a mean of 487.3 ± 43.1 facets (difference $= 193.0 \pm 51.9$). Wild type disks in wild type hosts, pupating 24 hours after transplantation, formed a mean of 489.1 ± 28.8 facets; in *Mw* hosts, pupating in 68 hours, they formed 599.5 ± 23.1 facets (difference $= 110.4 \pm 36.9$). The following conclusions may be drawn: 1) The length of the interval between transplantation and puparium formation of the host, during which the transplant increases in cell number, is a major factor in determining the size attained by the transplanted disk. This is in agreement with data on Bar obtained under different conditions by Bodenstein. 2) Increase in cell number continues in the wild type optic disk after 76 hours (data from + in + and + in *Mw*). 3) Under conditions of the experiment, the wild type disk forms fewer facets in transplant than in situ.

BRINK, R. A., and COOPER, D. C., University of Wisconsin, Madison, Wis.: *The significance of double fertilization in flowering plants.*——The early collapse of fertile ovules in *Medicago sativa* is about five times as frequent following selfing as after cross-pollination. This type of seed failure, which is termed somatoplastic sterility, is shown to be due primarily to excessive meristematic activity of the inner integument, causing starvation of the endosperm and embryo. Continued development of the young seed apparently requires that the endosperm maintain an ascendant physiological position relative to the growing maternal tissue, (nucellus or integument, as the case may be), adjacent to the embryo sac with which it must compete for a limited food supply. Otherwise the partition of nutrients becomes unbalanced in favor of the nucellus or integument, and somatoplastic sterility results. Marked heterosis is shown in the young hybrid endosperm, although it can not be detected in the much more slowly growing embryo. Conversely, inbreeding depresses the rate of nuclear division in the alfalfa endosperm, in many cases below the critical level for ovule survival. Double fertilization, therefore, may be interpreted as a mechanism which, through heterosis, enhances the competitive position of the endosperm in the delicately balanced internal environment of the ovule.

BRYSON, VERNON, Columbia University, New York.: *The modifying effect of Minutes.*——A wide variety of mutant genes have been combined with $M(3)$ and its allele $M(3)Fla$, with the purpose of determining the effect of extreme Minutes on dominance, penetrance and expressivity. Particular interest is attached to experiments involving B^iB^i, Bx^3, *co*, *fa*, ap^4, *Jag*, *Ser*, *Lyra* and *Xa*, combined individually with these Minutes. Here the modifying action of Minutes was well marked, whereas in numerous other cases no appreciable modifying influence could be detected. It is shown that Minutes often enhance the phenotypic expression of genes with which they are combined and that such modification is consistent with the generally accepted viewpoint that dominance, penetrance and expressivity are aspects of the same problem. In the heterozygote detection of modification involving any of these phenomena is correlated with the expression of one, as contrasted with two doses of the gene, in the non-Minute genotype. Enhancement must obviously be in the direction of the homozygote; e.g., *Jag/MFla* is phenotypically similar to *Jag/Jag*, not to *Jag/+*. Entirely consistent is the manifestation of phenotypic effect by confluens, which in the non-Minute heterozygote is undetectable, but in the Minute appears as though homozygous. Similarly penetrance may be increased. Modification of the homozygote is also in the direction of enhanced expression which may now be recognized as beyond the limits imposed by the normal residual genotype. If the homozygote is lethal but has a phenotypic effect in the heterozygote, then its heterozygous combination with Minute may fail to survive, as in *MFla/Dfd* (Payne). These experiments indicate that Minutes may be regarded as among the most effective of nonspecific modifiers in Drosophila.

COOPER, D. C., and BRINK, R. A., University of Wisconsin, Madison, Wis. *Somatoplastic sterility as a cause of seed failure following interspecific hybridization.*——Fertilization occurs between 50 and 60 hours after pollination in the following matings: *Nicotiana rustica* × *N. glutinosa*, and reciprocal, *N. rustica* × *Petunia violacea* and *N. glutinosa* × *P. violacea*. Seed is not formed, however, due to early collapse of the fertile ovules. Histological changes leading to failure are essentially like those frequently observable in normally cross-pollinated *Medicago sativa* after selfing, as earlier described by the authors under the term somatoplastic sterility. Normal seed formation follows self-pollination in *N. rustica* and *N. glutinosa*. Fertilization occurs 20–24 hours after pollination, and endosperm and embryo develop rapidly. Nucellar cells become flattened, forming a thin and gradually shrinking layer between endosperm and integument. The course of development following interspecific and intergeneric hybridization, on the other hand, is conspicuously different. The endosperm particularly, and possibly the embryo also, grow more slowly. The nucellus quickly becomes meristematic, instead of regressing, and forms two or more layers of cells. As the nucellar cells proliferate, starch appears in them, the endosperm breaks down and development of the ovule eventually ceases. The immediate cause of ovule collapse is attributed to endosperm starvation due to hyperplasia of the nucellus. The latter condition is believed to arise when the genetic constitution of the endosperm is such that it fails to attain and maintain an ascendant physiological position relative to the adjacent maternal tissue which is also stimulated to development following fertilization.

CUMLEY, R. W., and IRWIN, M. R., University of Wisconsin, Madison, Wis.: *Immunological and geographic relationships among pigeon species.*——Using immunological procedures, notably the agglutinin-absorption technic, it was found that the species of pigeons from the Old World and those from the New World, when compared, represented two more or less distinct groups. Briefly, there appear to be antigens which are common to species of both the Old and the New World. Further, within the species of the Old World there are other antigens which set these species apart as a group from any of those in the New World. These components are shared by many , if not by all, of the Old World species. And then there are other antigens which set each species apart from every other species of the Old World. Likewise, the species of the New World have a group of cellular characters which are not found in those of the Old World, and which are present in most, if not in all, of the species of the New World. Also, each species has its own distinctive antigens.

CURTIS, M. R., and DUNNING, W. F.; Institute of Cancer Research, Columbia University, New York, N. Y.: *An independent recurrence of the blue mutation in Rattus norvegicus and observations on a mosaic of blue and its normal allelomorph.*——A recurrence of the blue mutation described by ROBERTS in 1929 is reported in a strain of pedigreed agouti black hooded rats. The foundation

stock of this strain was three rats obtained from Copenhagen in 1920. The known genetic constitution of the strain is *CC PP AA hh*. The first observed blue mutants appeared in the 15th brother × sister generation of Line 2331. Prior to this time Feb. 23, 1934 intense hooded coat color had been recorded for each of the observed 6059 rats of this strain of which 187 were direct ancestors and their full sibs and 504 were half-sibs of the direct ancestors of the mutants. Since the appearance of the mutation the collateral families have produced 3565 pure line intense colored progeny and thousands of hybrids none of which have shown the mutant character. The identity of this blue character with the one observed by ROBERTS has been established by CASTLE in crosses of the two stocks. A hooded female mosaic for blue and intense color has recently been observed in an F_2 population of 286 rats from a cross of intense hooded (*Dh*) by blue selfed (*dH*). Her pattern is 26 percent colored with a black area on the right side of the face and left side of the cape equal to about 10 percent while the remainder of the colored area is blue. She has had to date 16 blue progeny by a blue selfed male indicating that the mosaicism is somatic.

DAWSON, W. M., and KATZ, REUBEN, U.S.D.A., Bureau of Animal Industry, Washington, D. C.: *Preliminary report on variation in ability of dogs to master a multiple-choice situation.*——As a basis for later genetic studies an attempt is being made to find types of behavior that will provide a clear-cut discrimination in the intelligence of dogs. A modification of the "Hamilton Multiple-Choice Apparatus" is used in one of the tests. The dog is faced with the problem of finding which one of four doors is unlocked. In these tests many dogs developed a good system of seeking the right door which eliminated the repetitions that would otherwise have been expected. Pulis, Chows, Border Collies and F_1's of Puli × German Shepherd, Puli × Chow, and Puli × Border Collie, totaling 44 dogs, were tested. When dogs were scored on avoidance of repetition, a wide distribution of scores was obtained. On the basis of total doors tried in approximately 140 trials by each dog the percent of doors not repeated varied from 39 to 80. The mean for the 44 dogs was 56.8 ± 0.9. Average scores for the three largest groups were: Pulis, 54.3 ± 2.3; F_1 (Puli × German Shepherd) 62.5 ± 1.3, and F_1 (Puli × Chow) 55.2 ± 1.5. While the means of the first two groups appear to differ significantly the magnitude of the reliability coefficient of the test ($r = .82$) and the small number of dogs in each group make it necessary to do further work before drawing final conclusions. One litter (Puli × F_1 (Puli × German Shepherd)) in which the F_1 dam was selected for her very high score on this test (80), has an average score of 63.9 ± 2.0 indicating that the selection may have had some effect.

DEMEREC, M., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *A comparison between the X-ray induced and the spontaneous Notches.*——Salivary gland chromosome study of an unselected series of 27 X-ray induced Notches shows that 3 had the full complement of bands, in 7 cases one

band was missing, in 4 cases 2 to 5 bands were missing and in 13 cases more than 6 bands were missing. Similar study of a series of 10 spontaneous Notches shows 1 case in the first group, and 3 cases in each of the other three groups. The spontaneous series consisted of Notches kept in stock at various laboratories and obtained from them for this study. Since long Notch deficiencies have poor viability it is probable that in this series natural selection may have eliminated some of the long deficiencies. Such a possibility finds support in the genetic analysis by LI and BRIDGES of spontaneous Notches collected at random; it was found that 4 out of 25 involved the white locus as well and were thus long deficiencies extending to the left of Notch. In my X-ray series 7 out of 27 involve white, while in the spontaneous series only one out of ten is white, suggesting the working of natural selection in the case of the spontaneous series. When this is taken into consideration the two series show a striking similarity in the frequency distribution between the four groups differing in the length of the deficient segment.

DUNN, L. C., Columbia University, New York City: *Changes of dominance in the house mouse.*——The Brachyury (short-tail) mutation behaved as a dominant in the stock in which it was found by DOBROVOLSKAIA-ZAWADSKAIA. When outcrossed and backcrossed to several inbred stocks of *Mus musculus* and to *Mus bactrianus*, the expression of the mutation in the heterozygote was *decreased*, the degree of dominance modification depending upon the particular stock to which crossed. These results resemble those obtained with two stocks by GREEN. Additional observations do not indicate any marked effect either of modifiers or of heterosis upon the homozygote which regularly dies as embryo at about 11 days.——One normal stock (Bagg albino) which had been shown to have plus modifiers for Brachyury was tested with another short-tail mutation, *Sd*, of similar phenotype. In continued backcrosses to Bagg albino, the effects of *Sd* increased progressively, so that most heterozygotes are now tailless. No effect on homozygotes was noted. The same inbred stock thus modified two similar phenotypes in opposite directions. It is thus evident that dominance modifiers may have some specificity.

EIGSTI, O. J., University of Oklahoma, Norman, Okla.: *Effects of colchicine upon the nuclear and cytoplasmic phases of cell division in the pollen tube.*——The cytogenetical variations resulting from effects of colchicine upon nuclear and cytoplasmic phases of the division of the generative cell were studied by the microscopic slide method in *Tradescantia occidentalis* (n-6) and *Polygonatum commutatum* (n-20). A treated series consisted of the addition of colchicine to the cultural medium (sucrose-agar). In control cultures the double metaphasic chromosomes were arranged upon an equatorial plate from which the daughter chromosomes separate into their respective nuclei. The presence of colchicine inhibited the formation of an equatorial plate stage and prevented the complete separation of daughter chromosomes but did not preclude the

formation of nuclei or impede the development of pollen tubes. A variable number of nuclei was found in the pollen tube of the treated series. Cytological studies of the morphology and number of chromosomes present in nuclei formed thus revealed that both daughter chromosomes were present in the same nucleus. A detailed study of two nuclei in one pollen tube of *Polygonatum* disclosed that one nucleus contained 11 double chromosomes (22 daughter chromosomes) and the other had 9 double chromosomes (18 daughter chromosomes). Each nucleus formed in the presence of colchicine therefore is a cytogenetic entity unlike any other nucleus in the same pollen tube or unlike the nucleus formed after the usual division of the generative cell. The frequency of chromosomal breakage is higher in treated material which indicates that colchicine induces variations other than polyploid changes. The production of chromosomal breakage and the formation of nuclei containing both daughter chromosomes when pollen tubes are treated with colchicine provides the basis for multitudinous variations of a cytogenetic nature.

GILES, NORMAN, Harvard University, Cambridge, Mass.: *Spontaneous chromosome aberrations in Tradescantia.*——The types, frequency, and loci of spontaneous structural chromosome aberrations in a number of pure species and in the progeny of a natural species hybrid of Tradescantia have been studied at first post-meiotic mitosis. These aberrations are similar in most respects to chromosome alterations induced by low dosage X-rays in the developing microspore. This similarity suggested that the spontaneous breaks might be due to natural radiation. Considerable evidence is presented, however, to show that this is not the case. Localization of the breaks in the centromere region suggests that tortional strains occurring during the coiling cycle may be a major factor in causing spontaneous breaks. The frequency of spontaneous aberrations varied widely among individuals of the F_2 generation, indicating the possible genotypic control of the aberrations. The average percent of breaks in the hybrids was about three times that of the pure species, and it is suggested that this increase may result from the recombination of genetic factors following hybridization.

GLASS, H. B., Goucher College, Baltimore, Md.: *Differential susceptibility of the sexes of Drosophila to the effect of X-rays in producing chromosome aberrations.*——Treatment of mated females of *D. melanogaster* by X-rays provides simultaneous exposure of sperms and pre-meiotic eggs to identical doses. Offspring from eggs laid in the first five days following treatment yield approximately equal frequencies of lethal mutations in ♂ and ♀ germ-cells. 55 translocations were all produced in ♂ germ-cells. This disproportion is significantly greater than would be expected from the elimination of translocations in eggs undergoing meiosis. Inversions have been obtained from both ♂ and ♀ germ-cells. They are more frequent in the former, but the significance of the difference is not yet established.

GLASS, H. B., Goucher College, Baltimore, Md.: *Genetic identity of translocations of independent origin.*——The genetic identity of translocations associated with the dominant mosaic eye-colors bw^{V3}, bw^{V4} and bw^{V5} in *D. melanogaster* has been proved by obtaining reciprocal homozygotes for chromosome 2L·3L from one origin and chromosome 3R·2R from another. The genetic test here is probably more critical than the parallel cytological studies of salivary gland chromosomes. These three translocations were derived independently from X-ray treatments. Their identity indicates the presence in the chromosomes of definite weak spots especially subject to breakage and rearrangement.

GORDON, MYRON, New York Aquarium, N.Y.C.: *Gene frequencies and parallel variations in natural populations of seven geographical species of Mexican fresh-water fishes.*——The members of the genera Platypoecilus and Xiphophorus are divisible into a number of geographical species; each species of a genus occupies a distinctive river system. Most of them display, in nature, a parallelism of heritable patterns. Most variable is *P. maculatus* with its 127 phenotypic expressions, mostly recombinations of 15 genotypes. Some genes are rare (*Sb*, *Có*, *Cò*, *Cs*, *Mc*, *Ct*, *T*), fairly common (*Sd*, *N*, *M*, *C*, *Co*) or quite common (*Sp*, *O*, *Cc*). In *P. variatus*, one subspecies has four genes (*Sp*, *M*, *C*, *Ct*), another has an extremely high percentage of one (*Sp*), and the third species has but one (*Sp*). *P. xiphidium* has four genes (*Sp*, *M*, *C*, *Ct*). Isolated populations within each species show a distinctive proportion of the genes. *P. couchianus*, furtherest north, has but the spotted gene (*Sp*). *X. montezumae* has a distinctive gene (*Sc*) in addition to *Sp* and *M*. *X. helleri* has the spotted gene rarely. *M*, *C* and *Ct* have been reported in the literature but are not represented in our wild caught specimens. A newly discovered pigmy Xiphophorus species has no patterns mentioned above. Collections made of *P. maculatus* in 1867, 1902, 1932 and 1939 indicate there is, in general, genic stability for a number of patterns. Comparative genetic studies of these patterns in all species is in progress.

GOWEN, JOHN W., Iowa State College, Ames, Iowa: *The structural significance of reproduction capacity in self-reproducing entities.*——In the evolution of complexity in living things the most striking phenomenon is the introduction of self-reproduction at some stage of the organization of high molecular weight proteins, between things called organic molecules and things called living. The characteristic reproductive capacity of a gene or a virus as well as size and mutative ability are common bonds of likeness between them. Inactivation of genes or of viruses, marked by loss of reproductive capacity, occurs in X-ray treatment. Tobacco mosaic virus of several strains may be inactivated exponentially by X-rays of 4 different wave lengths. By utilizing the quantum concept for the absorption of photoelectrons of known path lengths we may calculate the size of the vital volume of the molecule having to do with reproduction. This calculated volume is of 15,000,000 molecular

weight. Besides this volume, another portion of lesser but not as yet strictly defined size is occupied by elements capable of mutation without loss of reproductive capacity to the whole. The total of these two volumes presumably makes up the whole structure. An estimate of the molecular weight of this whole structure, 17,000,000 is furnished by the supercentrifuge experiment of SVEDBERG and ERICKSON-QUENSEL. A comparison of the two measurements shows that the portion of the molecule having to do with reproduction occupies the major part.

GREEN, E. L., and MCNUTT, C. W., Brown University, Providence, R. I.: *Bifurcated xiphoid, another effect of the short-ear gene in the mouse.*——A multiple recessive stock of short eared mice, $aabb(c^{ch}c^{a})ddpps^{e}s^{e}$, was found to have a formation of the xiphoid process of the sternum which differed from the normal rectangular type in that the posterior margin is deeply bifurcated. In F_1 from this stock by wild type (C_3H strain), the xiphoid processes were all rectangular. In the F_2 and the backcross generation, the bifurcated xiphoid reappeared in ratios characteristic of determination by a single mendelian recessive, though there was a slight but uniform deficiency of the recessive group. In every case, however, the progeny with bifurcated xiphoid were also short-eared. Since no crossing over between these two characters has yet occurred, it seems that bifurcated xiphoid process in this stock is caused by the same genetic mechanism which determines short-ears, and the morphogenetic processes which arrest the development of ears and bones of the cranium also arrest complete fusion of the bilateral sternal cartilages prior to their ossification in the medial plane to form normally the single rectangular xiphoid process. The deficiency of short-eared animals in segregating populations is probably caused by the often reported lowered viability of this genotype.

GREEN, M., and OLIVER, C. P., University of Minnesota, Minneapolis, Minn.: *The action of temperature and of non-allelic mutants upon heterozygous vestigial in Drosophila melanogaster.*——The frequency of wing notching in flies heterozygous for *vg* was not affected by 19, 23, 28, or 30°C, the frequency fluctuating between 0 and 1%. An increased frequency occurred with heterozygous *vg* in compound with dominant minutes. With $M(2)l^2$ the frequencies were 20, 65, 56, and 5 percent at the respective temperatures; and with $M(3)w$, 58, 80, 83, and 6 percent, with notching more extreme on the average. With each minute the highest frequency occurred at 23 or 28°. Several heterozygous mutant types were tested at 23° in compound with heterozygous *vg*. Each compound gave a notching frequency greater than that in the control, although in some cases only slightly higher. The frequencies varied from 1 to 82%, ranging in the order of increase: Controls; $In\ (3R)C$; *al S*; *Me*; *Pm dx*; $L^2M(2)l^2$; and $M(3)w$; but the degree of manifestation was not consistent with each increase in frequency. Some association between length of life cycle and frequency of notching is indicated, although that association seems not absolute. Temperature increase alone cannot account for the frequency

increase. Variations may be due to interaction of the mutant genes, possibly closely associated modifiers, with heterozygous *vg*, the temperature affecting the action of the modifying mutant. In general, males show a higher frequency of and also a more extreme notching. The notching varies from a slight to a beaded-like type.

HARNLY, MORRIS H.; Washington Square College, New York University, New York, N. Y.: *Experiments demonstrating no pupal critical period for wing size and form in four genotypes of D. melanogaster.*——Previous experiments with various genotypes for the vestigial locus have all shown a temperature effective period for wing size and form in the larva. It was thought that there might be another critical period for a different process of wing formation during the pupal stage, and that the second period might have a different critical temperature. Transferring dimorphos vestigial from 32°C at either 120 hours or puparium formation to 25° or 16° resulted only in a marked increase in mortality at the lower temperature. Our inbred vestigial stock was treated similarly and likewise gave negative results. Total development at 25° or transfers at puparium formation from 25° to 16° of the backcrossed vestigial-pennant stocks produced only flies with "nicked" wings. However, transfers at 96 hours to 16° gave the previously obtained frequency of normal and "nicked" wings. Tests of the original black vestigial-pennant stock produced at 16° only flies with normal wings, at 25° many with both wings normal, and at 30° some with normal wing margins. Furthermore, a "delta" was frequently present at 16° and extra cross-veins at the two higher temperatures. Preliminary reciprocal crosses between the two pennant stocks show that a sex-linked modifier affects the presence or absence of marginal nicks, and at least one or possibly two modifiers are associated with the appearance of extra cross-veins. A second critical period during the pupal stage was not found.

HEFNER, ROBERT A., Miami University, Oxford, Ohio: *Multiple alleles* (?) *in the inheritance of crooked little fingers.*——Earlier reports have designated hereditary crooked little fingers as Streblomicrodactyly. Subsequent investigations have revealed two additional types of hereditary factors resulting in crooked little fingers. These are given the descriptive titles of Minor streblomicrodactyly and Lateral streblomicrodactyly respectively. The former is shown from one family where it appears in 11 individuals. The latter is here shown from two families where many individuals are affected. The exhibit shows the three types of crooked little fingers by photographs, X-ray prints, and family charts. No family shown by these charts has any known relation to any other family in the group. The separate inheritance of each of these types of crooked little fingers shows Mendelian dominance over the normal condition. In some records the inheritance is that of an irregular dominant condition. How any of the streblomicrodactylous conditions behaves toward others in the series is not known since families where more than one of the deviations has been introduced have not been discovered. The location of such combinations would be

of decided genetic interest. The assumption of *multiple alleles* is at this time purely hypothetical.

HUESTIS, R. R., University of Oregon, Eugene, Ore.: *Tests for linkage in Peromyscus.*——Tests of four coat colors with one another and with flexed tail in *Peromyscus maniculatus* show no proof of linkage in any case. A double recessive recombination class was not obtained in over 400 F_2 individuals produced from a silver agouti-flexed tail cross but the ratio of individuals in obtained classes does not fit a theory of close linkage. The non-appearance this recombination class is not explained.

HUETTNER, ALFRED F.; Queens College, Flushing, N. Y.: *Differentiation of the gonads in the embryo of Drosophila melanogaster.*——After the primordial germ cells have been shifted dorso-posteriorly, they enter the amnio-proctodaeal invagination, where they remain loosely clustered at the posterior end of the diverticulum. They stay there impassive for approximately two hours without undergoing mitosis. During the sixth hour of incubation at 25°C, they make their way into the body cavity through the columnar cells of the gut diverticulum. This movement cannot be seen in the living material. From the study of fixed preparations, the impression is gathered that the movement through the gutwall is essentially amöeboid. As the hindgut diverticulum grows backward, the mesentodermal cluster of cells associated with the gut is carried dorso-laterally, where these cells will later contribute to the formation of the dorsal wall of the midgut. It is during this movement that the primordial germ cells are carried to their permanent position and become surrounded by mesentodermal cells. In ten to eleven hour embryos the gonads are formed. Not all the primordial germ cells which are carried passively into the amnio-proctodaeal invagination in the early embryo, are incorporated into the gonads. Some of them never pass through the gutwall into the body cavity, in others the migration is never completed, and in other instances they become entangled and lost in the yolk mass. Counts of the number of primordial germ cells in the primitive gonad show that approximately five to seven are grouped in some, whereas ten to thirteen are observed in others. In the light of KERKIS' report of the size differences in the gonads of newly hatched male and female larvae, these observations indicate that the sex of the gonads may, in all likelihood, be determined in the ten to eleven hour old embryo.

HUMPHREY, L. M.; University of Arkansas, Fayetteville, Ark.: *A preliminary report of the effects of inbreeding in cotton with special reference to staple length and lint percentage.*—In 1936 the inbreeding and hybridization method was introduced into the cotton improvement program at the Arkansas Experiment Station, and inbred lines were started in three varieties of cotton. Inbred lines for experimental purposes have been carried since 1930. In 1937 inbreeding was started in five more varieties and in 1938 in two more. All cotton varieties studied were found to be very non-uniform, particularly in fiber characteris-

tics. A highly significant negative correlation between staple length and lint percentage was found in five varieties, and no significant correlation was found in the remaining five. Cotton is largely self-pollinated, but there is enough cross pollination to introduce a certain amount of heterozygosity. Inbreeding rapidly segregates numerous types which become relatively very uniform after three to four generations, the inbred lines being much more uniform in all cases than the varieties from which they arose. Statistical studies of the lint percentages and staple length would indicate that the inbred lines become highly uniform after three to four generations of inbreeding, and no further significant increase in uniformity is evident up to eight generations. This would indicate the possibility of using three- or four-year inbred lines as inbred strains or varieties.

HUTT, F. B., and LAMOREUX, W. F., Cornell University, Ithaca, N. Y.: *A map for six linkage groups in the fowl.*——A map is presented illustrating the 23 mutations listed below, together with their wild-type alleles, and showing the approximate loci of 21 of these mutations in the six linkage groups now established for the fowl. These groups are numbered below in the order of their discovery and the numbers used have no relation to specific chromosomes. The approximate cross-over distances are given. (1) Sex chromosome: head streak—13.5—barring and slaty shanks (as yet unseparated in linkage tests) —27—brown eye—20—light down—16—silver—11—slow feathering. Locus of sex-linked gene for naked is not yet determined. (2) Rose comb—0.4—creeper—30—uropygial. (3) Crest—12.5—dominant white—17—frizzling, with fray tentatively assigned to this group. (4) Blue egg—5—pea comb—33 —marbling—46—naked neck. (5) Silkie—10—flightless. (6) Duplex comb—28—multiple spurs. In the sex chromosome arrangement and distances given, present what seems the best fit of all available data. In the second and fourth groups the distances actually determined are as given but the arrangement requires confirmation.

HUTT, F. B., Cornell University, Ithaca, N. Y.: *A new linkage group in the fowl.*——Genetic studies of the multiple spurs found in Black Sumatra fowls have shown this character to be an autosomal unifactorial mutation, incompletely dominant in heterozygotes but always easily distinguishable from the normal condition. The symbol *M* is proposed for the causative gene. Males have usually three spurs on each shank, but may have up to five. In adult females the mutation is clearly shown by three enlarged scales and an absence of the spur papilla usually found in females. It is classifiable in newly-hatched chicks regardless of sex with an accuracy of more than 98 percent. For these reasons the mutation is especially useful for studies of linkage. Tests for linkage with dominant white, rose comb, flightless and pea comb (each a marker for a separate linkage group) have all shown independent segregation of multiple spurs, thus apparently eliminating the gene *M* from the four autosomal linkage groups previously established. Further tests have shown it to

be linked with the gene *D*, for duplex comb, there being about 28 per cent of crossing over between the two genes. This establishes a fifth autosomal linkage group.

IRWIN, M. R., and COLE, L. J., University of Wisconsin, Madison, Wis.: *Interrelationships of the cellular characters of three species of Columbidae.*——That part of the antigenic pattern of the red blood cells of Pearlneck (*Streptopelia chinensis*), not shared by Ring dove (*St. risoria*), has segregated into at least ten different characters, following backcrosses to Ring dove of the species hybrid and selected backcross birds. These characters have been designated as d-1, d-2, d-3 . . . d-11. Each of these individual components is produced by the action of one or more genes. The results of various tests show that a part of the specific antigenic complex of Pearlneck, as contrasted with Ring dove, is shared with still a third species, the Senegal (*St. senegalinsis*). Of the ten specific components of Pearlneck, only two (d-6 and d-11) appear not to be shared *in toto* with Senegal. It would be expected, then, in a cross of Pearlneck and Senegal, the offspring of backcrosses of the species hybrid to Senegal would be of four kinds in approximately equal numbers: viz., those whose cells contain (1) both d-6 and d-11, (2) d-6 alone, (3) d-11 alone and (4) neither of these constituents. Each of these four types of cells has appeared in the backcross offspring. The actual results obtained following a genetic test of the relationship between these particular species gives added confidence as to the accuracy of similar comparisons between Pearlneck, for example, and other species with which no hybrids have been obtained.

IRWIN, M. R., and CUMLEY, R. W., University of Wisconsin, Madison, Wis.: *Interrelationships of the cellular characters of species of Columba.*——Earlier studies have revealed that in contrasting cellular characters of two related species, some characters are shared by both species, and, in addition, each species contains cellular components which are peculiar to itself. At least a part of the characters which distinguish one species from another may in turn be shared by a third. In both doves and pigeons, these species specific substances have been found to be genetic characters which segregate in accordance with Mendelian laws, and which are transmitted as unit substances through species-hybrid and back-cross generations. In the present comparison of eleven species of *Columba*, some pairs of species were found to possess the major part of their antigenic components in common; and their specific characters were but infrequently shared with other species. In other contrasts, two species were seemingly more distantly related, having less antigens in common and sharing at least a part of their respective specific antigens with a majority, if not all, other species. All intermediate degrees of relationship were found. Apparently, no one species is composed of a combination of the antigens (genes) of two other species, and it is doubtful whether a combination of antigens in any number of species could account for all the antigens present in any one. However, the data regarding this question are as yet incomplete.

JEFFREY, EDWARD C., and HAERTL, EDWIN J., Harvard University, Cambridge, Mass.: *Nuclear fusions in relation to chromosomal structure and inheritance.*——The demonstration is illustrated by photomicrographs, microscopic preparations and drawings. These show the great advantages in connection with the study of chromosomal organization resulting from the study of the numerous examples of nuclear fusions, namely reproductive (diploid) and endospermal (triploid and quadruploid) presented by the Angiosperms. In all cases regardless of the number of chromosomes involved the organization of the post-fusional chromosomes is the same as in somatic cells, namely each chromosome contains two oppositely spiraled chromatids. It is thus clear that there is no microscopically visible result arising as a sequal to nuclear fusions, reproductive or otherwise. The facts emerging in the present investigation depend on the utilization of a greatly improved technical manipulation in connection with the fixation and staining of the chromosomes, which results for the first time in a clear revelation of their internal organization. It is now obvious that the structure of chromosomes of the categories somatic (sporophytic), meiotic, gametophytic and trophophytic (endospermal) is identical. In other words chromosomes may differ in number in the various categories of nuclei but not in organization.——This situation appears to necessitate a profound revision of cyto-genetical theories related to chromosomes, particularly the long held hypothesis of synapsis (syndesis) or chromosomal pairing in meiosis. The terms leptotene, zygotene, pachytene and diplotene also apparently can no longer be logically or accurately used. Chiasmatypy, so called, is as characteristically present in somatic, gametophytic and endospermal divisions as in those designated meiotic and consequently can apparently have no significance in connection with reproductive inheritance.

KAUFMANN, B. P., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Induced changes in chromosomes carrying inverted sections.*——The distribution of 278 X-ray induced breaks was plotted by use of salivary gland chromosomes of *Drosophila melanogaster* containing the dl-49 inversion in order to detect any possible reinversion and to measure break frequency in regions adjacent to the limits of the inverted section. No significant differences were detected between break distribution in these regions and in similar regions of chromosomes having the wild-type sequence of banding. However, data for the entire X chromosome showed that there exist certain intercalary regions of high break frequency, which probably contain heterochromatic material. If the original breaks leading to the inversion should occur in such intercalary regions, the possibility of reinversion would be increased.——As a corollary of this study, analysis was made of a series of induced changes to wild-type from roughest^3, which is associated with a long inversion extending from 3C to near the spindle attachment region in the X chromosome. Reversion of roughest^3 is not infrequent, and five changes to wild-type have been analyzed. Two are reciprocal translocations between 2L and X heterochromatin; two are reciprocal translocations between 3R and X heterochromatin;

one involves transfer of the nucleolus-organizing region from the X to 3L. Obviously phenotypic reversion of roughest[3] may be associated with various types of chromosomal rearrangements, independent of reinversion, probably involving in each case a break or breaks in the proximal heterochromatic region of the X chromosome.

LAWSON, CHESTER A., Wittenberg College, Springfield, Ohio: *Sexual balance and the differentiation of aphid types.*——It is proposed that differentiation of aphid types is controlled by the opposing action of two forces (male and female) operative during development. If the male force dominates completely, a male develops. If the female force dominates completely, a gamic female develops. Incomplete dominance by the female force causes the development of the following types in order of decreasing female dominance: gamic-parthenogenetic female intermediate, wingless parthenogenetic female, wingless-winged parthenogenetic female intermediate, winged parthenogenetic female. Parthenogenetic females are sex intergrades or mosaics. Supporting evidence is found in the classification of differential structures into male and female characters and the correlation of these characters with aphid types. Male characters are wings, wing muscles, ocelli, many secondary sensoria on antennae, thin hind tibiae lacking sensoria, male reproductive system. Female characters are no wings, no wing muscles, no ocelli, few secondary sensoria on antennae, swollen hind tibiae with many sensoria, female reproductive system.

LAWSON, CHESTER A., Wittenberg College, Springfield, Ohio: *The probable mechanism controlling wing development in aphids.*——The germaria in embryonic winged and wingless aphids differ prior to time of determination of wings. Nurse cells in germaria of embryos destined to be wingless begin secretion of "nutrient" substance before wings begin development. This secretion presumably inhibits wing development. Nurse cells in germaria of embryos destined to be winged do not begin secretion before wings begin development. Lack of such secretion permits wing development.

LINCOLN, RALPH E., Iowa State College, Ames, and Cornell University, Ithaca, N. Y.: *Production and rate of mutation in Phytomonas stewartii by X-radiation.*——Broth cultures of *Phytomonas stewartii* were treated with X-radiation of 0.7A and 1.5A at intensities which reduced a culture of 100,000,000 viable cells to less than 1,000 viable cells within a period of 25 minutes. Inactivation curves for this organism are of the single absorption type——that is semilogarithmically linear. Variation was observed in colony type, colony color, virulence and mucoid characters. Mutation increases in proportion to exposure time. Under conditions where only one out of every 100,000 cells treated remains viable, the mutation rate is about 200 times higher than the natural mutation rate after growth in broth, or about 10,000 mutations per million treated cells as compared to 55 per million cells after

growth in broth. In general the same pattern and type of variation was found after X-radiation as after growth in broth. Most mutations observed are stable, hereditable and transmissible from parent to daughter cell, although certain stocks have been isolated in which it is impossible to stablize the variant type.

LOVE, R. MERTON, Cereal Division, Central Experimental Farm, Ottawa Canada: *A cytologically deficient speltoid of hybrid origin.*——Three bearded speltoids were found in F_2 Huron X R.L. 1005. Both varieties are bearded spring wheats. Progeny of the three speltoids totalled 36, of which 20 were normal and 16 speltoid. The 15 plants examined cytologically had 21 bivalents. Nine were normal cytologically and six were characterized by a heteromorphic bivalent, one member of which was deficient for the longer arm. Phenotypically, the nine plants were normal (in respect of the speltoid characters) and the six plants heterozygous for the deficiency were speltoids. No individuals homozygous for the deletion were obtained. Although the number of plants was small, the ratio seems to be characteristic of the C series type. The occurrence of the aberrant plants emphasizes the importance of hybridization in the origin of off-types. Breaks at the attachment region in unpaired chromosomes are a fruitful source of deletions which result in certain specific off-types in *vulgare* wheats. Hybridization, accompanied as it is by a comparatively high proportion of univalents in F_1 and some plants of later generations, plays an important role in the origin of speltoids and other off-types characterized by visible deficiencies. At the same time most, if not all, hybrids between *vulgare* wheats are heterozygous for one or more inversions (unpublished data). Fragmentation, caused by crossing over in the inverted regions, results in deletions. This too will lead to off-types, but small deficiencies may not be detected in wheats, where prophases are difficult to study. It is quite possible that A-series speltoids fall in this category.

MYERS, W. M., U. S. Regional Pasture Research Laboratory, State College, Pa.: *Tetrasomic inheritance in Dactylis glomerata.*——The regular occurrence of quadrivalents at meiosis in *Dactylis glomerata* indicates that tetrasomic inheritance should obtain. When self-pollinated, one plant produced normal green and chlorophyll deficient (lethal) seedlings in a ratio approximating 35:1. In progeny tests of 63 first generation inbred plants 16 produced only normal seedlings and 47 produced both normal and chlorophyll deficient seedlings. By means of X^2 the segregating families were separated into 32 approximating 35:1 and 15 approximating 3:1 ratios. Assuming that the parent plant was duplex (*AAaa*) with the gene located near the spindle fiber attachment, the expected ratio is 16.2 not segregating, 32.4 segregating 35:1, and 14.4 segregating 3:1. X^2 for fit of observed to calculated gave a value of P above .99.

LUCE, WILBUR M., University of Illinois, Urbana, Ill.: *Effects of oxygen on development and facet number in a bar infrabar strain of Drosophila melano-*

gaster.——Preliminary observations by MARGOLIS and by myself have indicated that oxygen has considerable effect on mean facet number and on the length of the period of development in bar strains of Drosophila. In the experiments here reported, an inbred strain of bar infrabar was subjected to treatment with oxygen for periods varying from 6 to 96 hours at different times throughout the egg, larval, and pupal stages. Continuous treatment throughout the entire egg stage or the entire pupal stage had little effect either upon the speed of development or upon mean facet number. Due to its lethal effect prolonged treatment could not be used during larval stages. Here treatment varied from 6 to 24 hours. Speed of development during larval stages was retarded by the treatment, the greatest retardation resulting from treatments during the first, second, and the first half of the third instars. Treatment during late third instar and prepupal stages had a slight retarding effect. The period of greatest effect on facet number was found to be from the 60th to the 80th hours of development, which corresponds closely to the temperature effective period for facet number in this stock. Oxygen has less effect on facet number in bar infrabar than it has in bar or infrabar. Thus the treated females showing the greatest effect had a mean of 31.18 ± 0.49 compared with controls at 24.88 ± 0.32.

MCNUTT, C. W., TETREAULT, T. E., and SAWIN, P. B., Brown University, Providence, R. I.: *Hereditary variations in the vena cava posterior of the rabbit.*——In most mammals, including the rabbit, the posterior vena cava typically lies to the right of the dorsal aorta and passes dorsal to it at its bifurcation into the two common iliacs. This position in man, cat and pig is due to the persistence of the embryonic right supracardinal vein as the major part of the adult posterior vena cava, whereas deviations from it represent a substitution of one or another of the posterior cardinals, supra cardinals or cardinal collateral canals. A preliminary survey of these variations in five inbred families of rabbits and in crosses between them discloses approximately ten percent of so-called anomalies, among them being the marsupial type in which the vena cava, throughout its length and at its bifurcation, lies ventral to the aorta, representing a persistence of the embryonic cardinal collateral canals. Although the incidence of these variates in the total population is small and occurs in all families in which any numbers have been examined, it is found that ninety percent of them are either in inbred family V or in cross-bred matings to which family V contributes, and that within this family the most common variate is the marsupial type which in every case traces back two generations to the same male ancestor. The apparent genetic relationship between internal morphological characters peculiar to two separate but closely related orders of mammals suggests the possibility that further study of this variation may bear upon processes of phylogenetic as well as ontogenetic significance.

METZ, C. W., Department of Embryology, Carnegie Institution, Baltimore, Md.: *The nature of the chromatic granules in salivary gland chromosomes.*—— The range in pattern variation in selected regions is illustrated. For example,

in *Sciara ocellaris* one short region may appear in any of the following configurations: (1) Four conspicuous, similar transverse bands. (2) Three bands, the middle one much denser than the others. (3) Two transverse rows of conspicuous, block-like granules with concave sides, the granules of one row being much heavier than those of the other, and alternating with them in checker board fashion. (4) One row of block-like granules, with only faint traces of any additional structure. The block-like granules seem clearly to owe their concave sides and checker board arrangement to the presence of large achromatic droplets lying between them. The evidence here agrees with other evidence in indicating that the granules are chromatin masses which arise through distortion of the disks by the achromatic droplets, and that they have *per se* no genetic significance.

METZ, C. W., Department of Embryology, Carnegie Institution, Baltimore, Md.: *Spontaneous chromosome breakage during embryonic development in Sciara.*——In *S. reynoldsi* one chromosome pair regularly breaks into two segments during development in the salivary glands but not in ordinary embryonic cells. This provides additional evidence that the salivary gland chromosomes are highly modified structures. Here, as in the somewhat similar case described previously in *S. ocellaris*, the break probably occurs at the centromere region. It is postulated that two centromeres are present and that they separate spontaneously to effect the break. The phenomenon may have a bearing on the mechanism of evolutionary change in chromosome number.

MURRAY, M. J., Cornell University, Ithaca, N. Y.: *An F_2 study of interspecific characters.*——*Acnida tamariscina* (Nutt.) *Wood* native to the western prairie states hybridizes readily with *Acnida cuspidata* Bert. native to the coastal plain from Florida to Louisiana. F_2 and backcross progenies indicate that the major genetic differences between these two species are of three types: 1) On the basis of pollen abortion chromosomal dissimilarities are postulated; 2) Color and certain characters of the female flowers are inherited as simple Mendelian differences, while 3) such differences as leaf shape and size, stem size and character are inherited as quantitative characters. The chief differences involved in speciation in this particular example are of a quantitative nature presumably due to multiple genes. Hybridization is prevented only in so far as geographical and ecological barriers are effective.

MURRAY, M. J., Cornell University, Ithaca, N. Y.: *An attempt to obtain sex-linked mutations in a dioecious plant.*——A normal female plant of *Acnida tamariscina* (Nutt.) Wood was pollinated with X-rayed pollen. Seed from 474 different F_1 open-pollinated females were grown. No visible sex-linked mutations were found in over 250,000 F_2 seedlings and approximately 10,000 mature F_2 plants. This suggests that the Y chromosome has a full complement of allelomorphs. However, ten chimeras were obtained. These were chlorophyll changes associated with fifty percent bad pollen in the affected areas.

MYERS, W. M., and HILL, HELEN, D., U. S. Regional Pasture Research Laboratory, State College, Pa.: *The association and behavior of chromosomes in autotetraploid grasses.*——Three plants of *Dactylis glomerata* had a range in mean quadrivalent frequency in microsporocytes of 3.3 to 4.2, an average of 3.9; five plants of *Agropyron cristatum*, 3.4 to 4.2, an average of 3.7; and nine plants of *Arrhenatherum elatius* 3.4 to 4.6, an average of 3.8. The maximum number possible in each species is 7. The frequency of sporocytes with lagging univalents at anaphase I varied from 3.0 percent to 66.0 percent and generally corresponded in different plants with the frequency of their metaphase I nuclei showing univalents. In most plants there was an excess of laggards, indicating that some of them originated from other sources than metaphase I univalents. The lagging univalents divided equationally at anaphase I, and a majority of the half chromosomes were included in the daughter nuclei; the remainder formed micronuclei. The percentage of quartets showing micronuclei was correlated in the different plants with the frequency of lagging univalents at anaphase I. Observed numbers of micronuclei in eight plants varied from 13 to 52 percent of the numbers expected if all of the daughter half chromosomes from the anaphase I laggards formed micronuclei. One plant had more than was expected. The number and position of micronuclei in the quartets confirmed the assumption that most, if not all, of them originated from lagging and dividing univalents at anaphase I. Chromatin bridges and acentric fragments at anaphase I and telophase I of eleven plants studied indicated that each was heterozygous for one or more inversions.

NABOURS, ROBERT K., and STEBBINS, FLORENCE M., Kansas Agric. Exp. Station, Manhattan, Kan.: *Chromosome aberrations and viability in Apotettix eurycephalus Hancock.*——The X-ray induced translocations of chromatin have been reported, one with the exchange between first and fourth autosomes, with depletion in amount of chromatin for the former and augmentation for the latter (1933); the other between a first autosome and a sex-chromosome, with increase in chromatin and probably an extra traction fiber attachment for the latter. The autosome of this translocation was lost (1935 and unpublished). The three surviving aberrators are designated tentatively as 1. donor(a1), 2. receptor(a4), and 3. receptor(X). The genetic and cytological data correlate (Robertson, 1935, and unpublished). Zygotes carrying donor(a1), and otherwise normal, hatch but rarely survive to 3-4 instar when they are recorded (14 donor(a1): 2443 normals). Receptor(a4) are 13 percent, and receptor(X) 14 percent, less viable than controls. Donor(a1) +receptor(X) are 38.5 percent below normal. Donor(a1) +receptor(a4) +receptor(X) fall 17 percent below controls. The data comprise approximately 50 other items of comparison of 15,088 aberrators with 49,000 normal, and with each other. In practically all cases augmentation of chromatin is accompanied by less mortality among males than females, and vice versa when there is a depletion of chromatin. There is indication, but not yet definite proof, that the XO(absence of Y) chromosome condition in grouse locusts is responsible for the significantly

lower viability, and perhaps for the much smaller size, of the males among the controls in grouse locust breeding.

OFFERMANN, CARLOS A., University of Chicago, Chicago, Ill.: *Interference and the mechanism of crossing over.*——The present study discloses the general character of interference which, in view of the evidence offered, should be regarded as the expression of some intimate chromosomal feature uniformly manifest in normal and rearranged chromosomes. Numerous tests concerning the X-chromosome of *Drosophila melanogaster* led to the conclusion that in the euchromatic region of a chromosome, total obstruction of crossing-over at any point changes linkage relations according to a definite law: crossing-over will be restored with increasing distance from the point of interference at a rate approximated by $y = 1 - e^{-kx^4}$, where y represents the coincidence value at a distance of x map units from the point of interference. k has a fixed value for a given set of conditions depending upon age, temperature, irradiation etc. Thus for normal temperature (25°C) $k = 715$, and for high temperature (31°C) $k = 2500$, taking the age average for the first six days. The new formulation of coincidence in terms of distance opens up the possibility for universal comparison of different types of interference given hitherto only separate and incomplete consideration. Closely resembling pictures based on extensive tests for a given temperature are revealed for interferences resulting from transposed spindle attachments in homozygous and heterozygous condition, heterozygous rearrangements, combined rearrangements, and multiple crossovers in the normal chromosome. A substantiating check is offered by the parallelism displayed in each case by both the normal and the heat series. The unit concept thus established entails a series of conclusions concerning the mechanism of crossing over; linkage relations in new rearrangements will be predictable.

PARKER, MILTON M., Ohio State University, Columbus, Ohio: *The rôle of constitution in the emotionality of the adult albino rat.*——The principle objective of the present experiments was to determine the extent to which emotionality might be the characteristic product of a constitutional basis, and the extent to which it might be influenced by non-constitutional or environmental factors. The reasoning was that if a valid measure of emotionality were available, and were applied to a genetically heterogeneous stock of animals raised under uniform conditions, then intercorrelations obtained between emotionality measured in several different situations should afford an estimate of the variance attributable to constitutional as well as environmental factors. The measure of emotionality involved the ability of the rat to inhibit defecation in repeated presentations of an emotion-provoking situation. The emotionality score was equal to the number of trials which provoked emotional defecation until three successive trials failed to provoke defecation. Six emotion-provoking situations were constructed. Each situation administered a different type of intense stimulation and required a different mode of adjustment. The measure of adjustment, however, always consisted of the cessation of defeca-

tion. Standardization, on the basis of odd versus even trials, showed the reliability for each situation to be not less than 0.85. The fifteen intercorrelations (for each sex) obtained between various combinations of the six situations were all beyond the "one percent point" of significance, the coefficients varying from 0.65 to 0.85. The consistency and significance of the results suggest that emotionality is quite characteristic of the individual organism, although it may obviously be affected by environmental influences.

PATTERSON, J. T., STONE, WILSON, and GRIFFEN, A. B., University of Texas, Austin, Tex.: *The virilis complex in Drosophila.*——This complex can be broken down into two groups. One of these groups is more closely related to *D. virilis virilis* (Spencer) and includes, at the present time, the standard *virilis* from Pasadena, strains from New Orleans, China and Japan, and several Texas strains. The other group includes *D. virilis americana* (Spencer), *D. virilis texana* (Patterson) and at least one other Texas strain. The salivary gland chromosome analysis shows that all members of the *virilis* group are very similar; the members of the *americana-texana* group differ from the *virilis* group and among themselves. The metaphase chromosomes vary within the complex from five pairs of rods and one pair of dots to one pair of rods, a pair of dots and two pairs of V-shaped elements. Tests show that cross-fertility is very high in the *virilis* group, rather high in the *americana-texana* group, but low between the two groups. Genetic tests show that different cross-sterility factors exist in the several strains, and that at least some of these factors are recessive. There are several morphological and physiological differences between the several strains. However, sex ratios in the crosses are normal; both males and females are fertile, although there are some peculiarities in the fertility of F_2 and F_3 generation males bearing an *americana* or a *texana* Y-chromosome. The hybrids are usually normal. From these facts we infer that the genic balance and the sex-determination mechanism are much alike in the two groups.

PHILLIPS, H. M., Emory University, Emory University, Ga.: *Karyotaxonomy of Erythronium.*——1. *Preliminary survey of chromosomal number, morphology, and structure of developing microgametophyte of americanum.*—— In addition to morphological characters so often used, recent advances in systematics have introduced methods of observing additional natural phyletic indices. Chromosomal numbers, chromosomal morphology and structure, and genic changes have been used as a basis for verification or disagreement with systematists. Phyletic divergencies, families, genera, and species, have been shown to be correlated definitely with chromosomal changes. A preliminary investigation of *Erythronium americanum* has indicated the possibilities of this group for a detailed comparative study of hybridization, speciation, and phylogeny. An attempt will be made to determine the correlation existing between these phenomena and the karyology of the genus since the chromosomes

are large enough for a detailed analysis of structure and structural changes. Material is abundant, and the plants exhibit a number of interesting morphological variations. Material was collected from plants growing on Mt. Pinola near Atlanta, Ga. For permanent smears CRAF fixative was employed. Temporary mounts were made with Belling's iron-aceto-carmine method and with several modifications of this method. The n-number of *E. americanum* is 12. Counts were determined at late prophase, metaphase, and anaphase. A survey of polar view metaphase plates gave following results: 3 with 11 chromosomes; 187 with 12 chromosomes; 3 with 13 chromosomes; 1 with 24 chromosomes; 1 with 36 chromosomes. These counts from the microspore divisions indicate the possibility of a polyploid series within the species. Of 25 plants selected at random, however, no heteroploidy was encountered. The chromosomes of the dividing nuclei fall into two general morphology types; five chromosomes with submedian centromeres and seven with subterminal centromeres.

RICK, CHARLES, M., Harvard University, Cambridge, Mass.: *X-ray induced chromosome deletions in relation to mutation rate in Tradescantia.*——Small spherical fragments, independent of other chromosome aberrations, result from X-radiation of Tradescantia microspores. Evidence from size and shape indicates most of these to be interstitial deletions of ring structure. Their frequency within the dosage range of 100 r to 600 r indicates that they are conditioned mostly by 2 hits and that they are produced at random. X-ray treatment at 3°C induces significantly more deletions than the same dose at 30°C. The effect of X-radiation during the interkinesis preceding and the one following meiosis on the size of subsequently developed pollen grains was studied. Variability following both treatments is significantly greater than in controls. Variability at low temperature exceeds that at high temperature. Pollen size is interpreted as, at least in part, genetically self-determined. Since minute deficiencies are inseparable in genetic behavior from point mutations, the close parallel between fragment frequency and variability in pollen size suggests that the latter is a measure of mutation rate.

RIFE, D. C., and BOYE, C. D., Ohio State University, Columbus, Ohio: *The genetics of certain leaf variations in Coleus blumei.*——The following genetic variations occur in *Coleus blumei*. Factor P extends purple throughout the entire leaf, and an allele p^G results in solid green leaves. A third allele p, results in leaves with a purple pattern on the upper epidermis, surrounded by a green margin, and a green lower epidermis. Both P and p^G are completely dominant to p, while plants of genotype Pp^G are a brown gray color. A dominant factor I results in a dark green chlorophyll, and its recessive allele in light green chlorophyll. P and I segregate independently. Another dominant factor A results in chlorophyll throughout the entire leaf, and its recessive allele in leaves with albino midrib. Dominant factor C results in crinkly leaves, and its recessive allele in smooth leaves. C and P segregate independently. Another

dominant factor S results in deeply lobed leaves and its allele in shallow lobed leaves. All observed plants of genotype *Ss* are male sterile. A second factor L also results in deeply lobed leaves and apparently is lethal in the homozygous state. Colchicine produces marked effects when seeds are immersed for twenty-four hours in a one percent solution. Seeds obtained by selfing purple plants of genotype *Pp*, when treated with colchicine, produce solid black, solid red and patterned progeny. Presumably black plants are of genotype *PPPP*, red plants *PPpp*, and pattern plants *pppp*. Black plants are self-sterile.

RILEY, HERBERT P., University of Washington, Seattle, Wash.: *Interaction of genes for flower color in Nemesia strumosa.*——Gene C determines anthocyanin pigment on the inside of the corolla lips; *cc* plants are yellow if gene W is present, otherwise white. The anthocyanin gene is linked with the self-sterility alleles with a crossover value of 37%. C plants vary from purple to red according to whether W and unidentified modifying genes are present. The deep purple "eyebrow" above the stamens and the numerous deep purple dots on the lower part of the throat are due to gene E; *ee* plants have just a few pale lines above the stamens and almost no dots in the throat. Homozygous f plants have a variable number of small purple dashes of flecks on the lower lips; this is apparently a mutable gene. Plants homozygous for *bl* have a light blue color on the upper lips which is absent in *Bl* plants. The *ee* condition is expressed only if no other anthocyanin pigment is present; *ee* plants that have gene C or genes *ff* appear phenotypically like E plants. In *cc ww blbl* plants there is a blue margin about three millimeters wide on the upper lips if E is present; in *cc ww blbl ee* plants, the blue covers the entire upper lips. In plants with C, *ww* and *blbl*, the margin on the upper lips is bluish-purple and the blue sap pigment appears to be precipitated by the purple and collected as granules in the center of the cells.

SATINA, SOPHIA, and BLAKESLEE, A. F., Carnegie Institution, Cold Spring Harbor, N. Y.: *Morphological differentiation in chromosomes of Datura stramonium.*——Length and structural differentiation of chromosomes were studied at metaphase stage of division in young pollen grains of 2n and 2n+1 plants. Pollen was chosen because only the haploid number of chromosomes is present. Half of the pollen grains from a 2n+1 primary type contain n+1 chromosomes, there being two of one kind, so that the extra chromosome could be identified. Half of pollen grains from 2n+1 secondary types also contain n+1 chromosomes but the extra chromosome is a doubled half-chromosome. Thus the ends of this chromosome could be identified. Use also was made of prime types when secondary types were unknown. Each chromosome in haploid complement is V-shaped, the insertion region dividing it into two arms of unequal size, the amount of difference depending upon the chromosome involved. The longest primary chromosome (1·2) averages 4μ; the shortest (23·24) averages 1.5μ. The longest secondary chromosome (1·1) averages

4.5μ, the shortest (19·19) averages 1.8μ. Seven of the twelve chromosomes have satellites on one of the arms. Five of the satellites are small. Both the ·4 end of the 3·4 chromosome and the ·10 end of the 9·10 chromosome have large satellites. Only one of the secondaries (10·10) has as its extra chromosome a satellited arm doubled. Differences in width of chromosomes also were observed. Comparison was made between chromosomes of pollen and root-tip cells of haploids. The small satellites were more conspicuous in root-tip cells.

SAX, KARL, Arnold Arboretum, Harvard University, Cambridge, Mass.: *X-Ray induced chromosome aberrations and their subsequent behavior.*——Both chromosome and chromatid aberrations are produced by X-rays. The frequency of aberrations produced by single breaks shows a linear relation to dosage, while those dependent upon two breaks increase as the square of the dosage. Many of these aberrations produce single or double chromatid bridges at anaphase. When these bridges break new fusions of broken ends occur, although some of the broken ends appear to behave as normal ends.

SEARS, E. R., U.S.D.A., University of Missouri, Columbia, Mo.: *Monofactorially conditioned inviability of an intergeneric hybrid in the Triticinae.*——Hybrids of *Aegilops umbellulata* with *Triticum monococcum* die at an early stage of growth, but when the same Aegilops species is crossed with *T. aegilopoides* (which on genetical and cytological grounds might be classified as a subspecies or variety of *T. monococcum*), the hybrids are viable. Results from both hybrid combinations are the same whichever direction the cross is made. Certain F_4 and F_5 derivatives of *T. monococcum*$\times$*T. aegilopoides* have also produced viable hybrids with *Ae. umbellulata*. The same derivatives have been crossed to *T. monococcum*, and individuals from the resulting F_1 and F_2 hybridized with *Ae. umbellulata*. The results indicate that *T. monococcum* carries a single, mendelian factor which has a dominant lethal effect whenever it is combined with a genom from *Ae. umbellulata*.

SHANK, D. B., Iowa State College, Ames, Iowa: *Top-root ratios of inbred and hybrid maize.*——Inbreds and single crosses of maize were grown for six weeks on the three treatments (1) compost soil (2) washed river sand low in nutrients and (3) water cultures containing full nutrient solution. Completely randomized blocks were used. Top-root ratios based on dry weights were obtained and the data were subjected to an analysis of variance. The ten inbred lines tested showed differences that were highly significant. Their means ranged from 2.0 to 3.1. Top-roots ratios of four F_1 generations were either significantly lower or did not differ from their low-ratio parent while in all cases they were lower than their high-ratio parent. No differences between reciprocal crosses could be demonstrated. Treatment differences were highly significant, with water cultures showing the highest, and sand the lowest mean top-root ratio.

SHULL, A. FRANKLIN, University of Michigan, Ann Arbor, Mich.: *Adult intermediate-winged aphids not physiologically intermediate.*——If intermediates

are due to development starting as of one type, but finishing as of another, the combination of characters shown would depend on which type of development preceded and on the order of determination. The observed combination of characters do not agree as to the order of determination, nor as to the direction of change; there are exceptions to any assumed order for either direction. Since the structures from which intermediacy has so far been judged are fixed and unchanging in the adult, the combination of them reflects only the processes occurring in the embryo. The adult might well be of one type or the other, not intermediate, in its physiology; and if so, it should be of the type toward which development was changing in the embryo. A number of adult intermediates in two strains of aphids have been tested as to wing production in their offspring, and as to the occurrence of males and gamic females among those offspring. Winged and wingless adults differ in these respects in both strains tested. The experiments indicated that adult intermediates were essentially like the wingless adults, indicating that the direction of change must be from winged to wingless. This agrees with histological observations which show that all aphid embryos, including the ultimately wingless ones, have wing rudiments before birth. It probably must be assumed that the change in development is not steady, but fluctuating.

SHULL, GEORGE H., Princeton University, Princeton, N. J.: *The gene mutations of Oenothera Lamarckiana and its mutational derivatives.*——Photographic presentation of gene mutations found in cultures derived from *Oenothera Lamarckiana* during the past 21 years. The first demonstrated gene mutation in the *Lamarckiana* series of forms was mut. funifolia, discovered in 1918. Older probable gene mutations were mut. *brevistylis* found by DE VRIES in nature, and mut. *rubricalyx* found by R. R. GATES in a mixed culture. In the 21 years since 1918 the following fully authenticated gene mutations have been discovered in the cultures of the exhibitor: In the first linkage group, associated with the zygote lethals characteristic of *Oe. Lamarckiana;* in order of their discovery, have been (a) mut. *funifolia* (1918); (b) mut. *pervirens* (1920); (c) mut. *rubrifolia* (1930); (d) mut. *pollicata* (1932); (e) mut. *clusa* (1934); (f) mut. *petiolaris* (1935); (g) mut. *contracta* (1938). In the third linkage group, and thus generally free from any indication of linkage with lethal factors: (h) mut. *vetaurea* (1921); (i) mut. *supplena* (1923); (j) mut. *bullata* (1925); (k) mut. *acutifolia* (1929). Not in the first linkage group, but with other linkage relations still unknown: (l) mut. *acuminata* (1936); (m) mut. *recurva* (1939); (n) mut. *rotundifolia* (1939). The last three are strikingly manifested in young rosette stages and appear in typical Mendelian recessive proportions in progenies grown from self-fertilized *Oe. Lamarckiana*, but they have not yet been brought to bloom. With exception of mut. *recurva* they are vigorous types and their rosettes persist throughout the first season of growth. Among the new gene mutations of the first linkage group, mut. *contracta* also has not bloomed and probably will never bloom. Its linkage relations are obvious because it completely takes the place of seg. *decipiens* in progenies of certain mut. *erythrina* parents.

SINGLETON, W. RALPH, and CLARK, FRANCES J., Connecticut Agricultural Experiment Station, New Haven, Conn.: *Cytological effects of treating maize pollen with ultra-violet light.*——A preliminary report of the cytologically observable effects of ultra-violet light has been published SINGLETON (1939). This is a continuation of the same study begun with DR. L. J. STADLER at the University of Missouri in 1937. Seed of five ears from treated pollen was grown (treatments and pollinations made by DR. STADLER). Three treatments with 2967 Å (56,000 ergs/mm^2) and one with 2967 Å (64,000 ergs/mm^2) produced 77 plants (from 84 seed planted). Of these there was one haploid, 19 plants with segregating pollen, 55 plants with normal pollen and two plants from which there were no collections. Seven of the segregating plants had no deficiency observable at pachytene; seven had observable deficiencies; one plant was very small and weak (no collection made) and was probably deficient; and four were not examined at pachytene. Three of the last four plants were normal at diakinesis but the fourth plant was not examined. Twenty-one plants (from 21 seed) were obtained from treatment with 2652 Å (28,000 ergs/mm^2). Two had segregating pollen, one with a deficiency while the other, even though segregating empty pollen, had no observable change at pachytene. Deficiencies observed were on chromosomes 2, 4, 6, 7 and 10. Two plants had a deficiency on chromosome 4. In addition there was one translocation, 3-armed, the plant being deficient for parts of chromosomes 1 and 10. There was also an anomalous case of an apparent deficiency for the long arm of chromosome 6 with the short arm present in triplicate. This may have been a sectorial as pollen from this plant was not segregating.

SINNOTT, EDMUND W., and WHALEY, W. GORDON, Columbia University, New York City: *The developmental basis of inherited size differences in plant organs.*——Measurements of diameter of stem, petiole, leaf blade, ovary and mature fruit in various pure lines of Cucurbita and Lagenaria show that these organs are correlated in size, a large-fruited type having all its vegetative structures also proportionally larger than a small-fruited one. These differences between races are all related to differences in the volume of the apical meristem of the shoot, which is markedly larger in large-fruited types than in small ones. In F_2 of crosses between large-fruited and small-fruited races, this same correlation between size of fruit and size of vegetative organs is evident, indicating that size differences of the various organs are not independently inherited, but that in a given individual they all have the same genetic basis. This evidently operates by controlling the size of the apical meristematic region, from which all these organs develop.

SKIRM, GEORGE, Arnold Arboretum, Cambridge, Mass.: *Frequency of quadrivalent formation in Tradescantia tetraploids.*——In Tradescantia tetraploids derived from diploid gametes most of the chromosomes pair as quadrivalents, but in tetraploids produced by somatic doubling most of the chromosomes pair as bivalents. This behavior is attributed to structural heterozygosity of inter-

stitial regions of otherwise homologous chromosomes. In a tetraploid produced by gametic doubling homology is not complete and there is no preferential pairing between the four "homologous" chromosomes. In tetraploids derived from somatic doubling there is complete homology between the duplicated chromosomes so that preferential pairing favors bivalent formation rather than quadrivalents. In cross pollinated plants with some structural heterozygosity of the chromosomes an autotetraploid produced by somatic doubling should be able to survive in nature, because it would have bivalent pairing and relatively high fertility from the beginning.

SONNENBLICK, B. P., Queens College, Flushing, N. Y.: *The salivary glands in the embryo of Drosophila melanogaster.*——The salivary glands in *Drosophila melanogaster* are ectodermal in origin. Transverse sections of embryos incubated for eight hours at $25 \pm .3$ C show them as a pair of latero-ventral ectodermal plates situated immediately adjacent to the ventral nerve cord in the anterior portion of the embryo. The ectodermal plates invaginate, and as they increase in depth an external orifice remains. In these early glands differentiation into duct and gland proper has not as yet occurred. Ducts have appeared in twelve hour old embryos. The lengthening ducts approach one another and in sixteen hour embryos have united medially to form a common duct which enters the floor of the pharynx. Examination of preparations of several hundred embryos indicates that no mitoses ever occur in salivary gland cells. From the time of the appearance of the gland as paired ectodermal plates through the period of differentiation in the later embryo no cytoplasmic or nuclear division has been observed. Increase in the size of the glands is due, therefore, to the growth of the component cells in the plates which initially invaginate to form the glands.

SONNENBLICK, B. P., Queens College, Flushing, N. Y.: *Dominant lethals and Drosophila embryonic development.*——The cytology of dominant lethal genetic alterations induced by X-radiation and their effects on Drosophila embryonic development have not, hitherto, been reported. Zygotes whose development is initiated by gametes bearing such alterations die at various levels of development prior to eclosion, but particularly striking is the high death-rate among the embryos. Study of the embryos has indicated that (a) dominant lethals, which can kill when present in single dose, are primarily large chromosomal aberrations; (b) effects of the lethals are evidenced at different stages of development, even as early as the initial cleavages; (c) normal and abnormal figures may be noted side by side in a single egg; (d) dominant lethals may be induced in both sperms and ova; (e) nuclei with irradiated chromosomes can multiply but, occasionally, undifferentiated cellular masses may be observed within some embryos; (f) death occurs in the diploid phase of development.

STILES, KARL A., and DOUGAN, PAUL K., Coe College, Cedar Rapids, Iowa: *A pedigree of malformed upper extremities.*——This study traces skeletal anoma-

lies through three generations. The defect varies greatly throughout the family. There are two individuals which have only a malformation of the fingers, namely, a webbing between the thumb and index fingers and an inward curvature of the little fingers. Seven individuals exhibit malformations similar to the above two cases plus a partial fusion of the radius and ulna bones of the forearm. Three extreme cases show not only deformities including the first two types, but also defects in the shoulders and upper arms. In two of these three cases the humerus is only about one inch long. The investigation includes four generations of twenty-six individuals, twelve of them showing some variation of the malformation. This skeletal morbidity is not inherited as simple Mendelian dominant, but rather irregular dominance is indicated.

SWANSON, CARL P., Harvard University, Cambridge, Mass.: *Heterozygous inversions in Tradescantia.*——The frequency of meiotic inversion bridges was studied in a hybrid population of Tradescantia. Inversion bridges were correlated with chiasma frequency. No correlation was found between bridge frequency and number of terminal chiasmata, but a very high correlation was found between bridge formation and frequency of interstitial chiasmata. Heterozygous inversions appear to be frequent in interstitial regions, but they are not involved in crossing over when chiasmata are limited to the terminal regions of the chromosomes. Chiasma localization at terminal loci permits both structural and genetic heterozygosity of the chromosomes in Tradescantia species with few meiotic irregularities. Genetic control of the loci of chiasma formation in hybrid segregates results in crossovers and inversion bridges in interstitial regions heterozygous for inversions. Thus chiasma distribution and chromosome inversions serve as isolating mechanisms.

WALETZKY, EMANUEL, University of Wisconsin, Madison, Wis.: *The disproportionate interaction of bifid with other wing mutants in Drosophila melanogaster.*——The mutant bifid changes wing venation markedly, but not wing size. When bifid is combined with some mutants of the scalloped wing type, disproportionately small wings are formed. The reduction in wing size exceeds that expected as a result of simple additive effects. Disproportionately small wings have been found in the combinations of bifid with various Beadex alleles, scalloped, clipped, or vg^{nipped}. They are absent in the combinations of bifid with cut^6, $vg^{vestigial}$, miniature, and radius interruptus.

WALETZKY, EMANUEL, University of Wisconsin, Madison, Wis.: *Correlations between the manifold effects of Wrinkled in Drosophila melanogaster.*——Reduced wings and abnormal black cephalic pigment are manifold effects of Wrinkled. The manifestation and the penetration of these two characters are correlated in flies heterozygous for Wrinkled. The smaller and more abnormal the wing, the greater the frequency and intensity with which the pigment appears. When the wings are practically normal, abnormal pigment is invariably absent. These relations also hold in $D+/+W$ and $D^3+/+W$ flies, which

frequently have practically normal wings. However, the penetration of the pigment character is not increased in $D+/+W$ flies whose wings are reduced by the presence of dumpy or miniature. W/W flies whose wings are very greatly reduced by $vg^{vestigial}$ show no abnormal pigment. Similarly, only a few Wrinkled vg^{nipped} and Wrinkled Beadex^{j} flies, with greatly reduced wings, show abnormal pigment.

WARMKE, H. E., and BLAKESLEE, A. F., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: *Polyploidy and the sex mechanism in Melandrium.*——Experimental polyploidy provides a new method for the investigation of the sex mechanisms in dioecious plants. The specific tools are: 1, the addition of entire chromosome complements; 2, the addition or subtraction of specific chromosomes by means of non-disjunction; 3, the synthesis of new types with intermediate sex chromosome constitution by segregation. In *Melandrium* (*Lychnis*) *dioicum* the diploid male may be represented as 2A+XY, and the diploid female as 2A+XX. The polyploid types 4A+XXXYY (male), 3A+XXY (predominantly male), 4A+XXXY (predominantly male), 4A+XXXXY (hermaphrodite) are therefore intermediate in sex chromosome constitution and give information regarding sex balance in this species. In the 4A+XXXY type it is possible to measure the chromosomes within a single cell and demonstrate that the Y chromosome is larger than the X. By utilizing a combination of the aids provided by polyploidy it is possible to locate the sex determining factors. Thus, the Y chromosome is shown to be male determining; it appears that the X chromosome is female determining, and that the autosomes play little if any role in sex determination. A 4n dioecious race, consisting of approximately equal numbers of 4A+XXXY males and 4A+XXXX females, has been established.

WATERS, N. F., and BRANDLY, C. A., U. S. Regional Poultry Research Laboratory, East Lansing, Mich.: *Studies in viability of poultry I. Inherent resistance to fowl paralysis.*——One of the objectives of the U. S. Regional Poultry Research Laboratory is to establish strains of definite genetic constitution with respect to resistance to so-called fowl paralysis. Experimental birds of ten different strains of White Leghorns were hatched from eggs purchased from breeders in widely scattered regions of the United States. Duplicate buildings and equipment on either side of a main Laboratory building are provided for the pathology and genetic studies. At the time of hatching, chicks of each strain from eight hatches were divided equally on a family basis. Approximately one-half of the chicks were placed on the genetic side and the other half on the pathology side, under a strict quarantine. Approximately two-thirds of the chicks on the pathology side were inoculated with blood from birds showing typical fowl paralysis, the others being held as contact controls. Examinations of birds from the genetic side affected with fowl paralysis as determinated by gross and preliminary microscopic observations show an incidence of 1.8 percent for the highest viable strain, while the least viable strain

had an incidence of 11.7 percent to November 1, 1939. Diagnoses of birds from the inoculated group showing fowl paralysis according to the criteria employed revealed an incidence of 11.8 percent for the most viable strain, and 30.4 percent for the least viable strain. Some families within each strain have shown definite resistance to fowl paralysis while other families have shown high susceptibility. There are a number of families in different strains that have had no mortality from any cause, while in some families from these same strains, mortality has been 100 percent. The data show results only through October 31, 1939. No definite conclusions of genetic significance can as yet be obtained from this material. There is the possibility that the resistance of a few of the families to fowl paralysis may be of genetic nature. There is, however, the possibility that such resistance may be acquired or that the birds may be latent or carrier cases of the disease.

WILSON, G. B., and NEBEL, B. R., New York State Agricultural Experiment Station, Geneva, N. Y.: *Changes in chromosome sensitivity to X-rays.*——Microspores of *Tradescantia reflexa* Raf. were rayed at various stages preceding first pollen-grain metaphase. Sensitivity was measured by the degree of chromosome alteration at first pollen-grain metaphase. Results indicate a positive correlation between the speed of mitotic development and the degree of response called "sensitivity." Several agents which either stop or retard division are being tested; first, for their own effect on chromosome and cell morphology and second, for their effects on sensitivity to X-rays as judged by comparisons with normal sensitivity curves.

WRIGHT, GERTRUDE, University of Toronto, Toronto, Canada: *Inheritance of form of flower in Linaria vulgaris Hill.*——Abnormal flowers found growing wild have afforded a starting point for this study. These are of two main types,——peloric and spurless-tubular. The extremes of both types show radial symmetry but flowers intergrading to the two-lipped normals are common. Pelorics and tubulars are recessive to the wild normals and the backcross gives in each case a 1:1 ratio. The F_2 of the cross normal $\times$ peloric is composed of 3 normal to 1 peloric. The cross peloric $\times$ tubular and its reciprocal produce normal flowers. That these synthetic normals differ genetically from the wild ones is shown by the crosses synthetic normal $\times$ peloric and synthetic normal $\times$ tubular. These result in the reappearance of both parental types in approximately a 1:1 ratio. Flower form seems therefore to depend upon at least two interacting factors. The wild normals may be designated *PPTT;* the pelorics *PPtt;* the tubulars *ppTT;* and the synthetic normals *PpTt*. If this is the case a new form is to be expected of the composition *pptt*. So far crosses of synthetic normals *inter se* have not revealed it. A few crosses of this type have yielded normals, pelorics and tubulars in the ratio 9:3:3 which suggests that the double recessive may be lethal. Further experiments are in progress.

ZELLE, M. R., and GOWEN, JOHN W., Iowa State College, Ames, Iowa: *On the origin of epidemic virulence.*——When an epidemic starts, a concatenation

of events occurs which enables a particular pathogen to sweep through a species. To particularize these events constitutes a most significant problem of disease resistance. If host and pathogen are closely integrated, one must change in susceptibility or the other in invasive power if epidemic disease proportions are reached. Following SCHOTT's experiments, our own have shown that susceptibility or resistance in different host populations may be markedly increased by 25 generations of controlled breeding. But these changes are too slow to explain the shift from a stable population to the unbalance of epidemics. The immediate origin seems rather in mutation of the pathogen's virulence. The selective force isolating and purifying the population to the invasive type would lie in the hosts.——Four experiments with mouse typhoid, *S. aetrycke*, support this analytical view. In experiments 1, 2 and 4 the host type did not materially influence virulence. The third experiment was markedly different. Bacteria, passed through resistant mice, suddenly became the most virulent experienced; those through the susceptible mice showed no such change. Reversal of hosts caused the very slightly virulent strain, previously inhabiting the susceptible mice, to gain virulence explosively in the third passage through resistant mice. The virulent strain from resistant mice, passed through the susceptibles, remained stable. The results thus far indicate infrequent pathogenic mutation with host selection as a cause of epidemic virulence, agreeing with work of WELLHAUSEN (1937) and LINCOLN (1939) on the corn bacterial wilt relationship.

EXPERIMENTAL EVIDENCE ON THE PRODUCTION OF THE MUTANT "ARISTAPEDIA" BY A CHANGE OF DEVELOPMENTAL VELOCITIES

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WERNER BRAUN[1]

University of California, Berkeley, California

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THE recessive mutant aristapedia (ss^a) of *Drosophila melanogaster* was first described by BALKASHINA (1929). It represents a remarkable case of homoeosis, in which the bristle on the antenna (arista) is transformed into a homologous organ, a tarsus (figure 1a and 1b). BALKASHINA has shown that in aristapedia flies segmentation of the antennal disk starts at two and one-half days of larval age, at the same time that segmentation of the leg disks begins, while in normal flies the segmentation of the antennal disks does not start until four and one-half days of larval age. GOLDSCHMIDT (1938) tried to explain these data in terms of developmental physiological processes. He assumed that the action of the ss^a gene consists of shifting the initiation of an embryological process to a different point in the time of development. He explained that if an evocator which determines leg segmentation is present in the germ at two and one-half days of larval age all disks in the proper stage of development will react to this stimulus by formation of a tarsus. The antennal disk of normal flies is far behind in differentiation at this time and, therefore, will not react to this evocator. According to BALKASHINA the differentiation of the antennal disk of ss^a starts at two and one-half days. This suggests that the differentiation of the ss^a antennal disk is speeded up and that it is so mature at the time when a "leg evocator" is present that it will react simultaneously with the leg disk in starting tarsal segmentation.

In 1937 two sets of experiments were started by the present author in order to bring forward experimental evidence for this view. The first part of these investigations was based on the consideration that if GOLDSCHMIDT'S explanation is correct, a combination of ss^a with different mutants affecting leg structure should exhibit the mutant effect on the antennae of aristapedia as well as on the legs. On the other hand, mutants influencing the arista should show no effect on aristapedia. The second part of the investigations attempted to prove the existence of a difference in developmental velocities by transplanting ss^a disks into normal larvae and normal antennal disks into ss^a larvae.

A short summary of the results obtained was published recently (BRAUN 1939). A more extended discussion will be given here.

[1] Assistance rendered by personnel of Work Projects Administration Official Project No. 465-03-3-192 is acknowledged.

COMBINATIONS OF ss^a WITH DIFFERENT LEG AND ANTENNAL MUTANTS

For the crosses involving mutants which affect the leg structure the recessive mutations dachs (*d*) and thickoid (*tkd*) were chosen. They are both located in the second chromosome. Dachs reduces the segments of the tarsus and results in shorter legs which are held close to the body. Thickoid exhibits especially short legs. *tkd* and *d* flies were crossed to ss^a flies, the factor for which is located in the third chromosome. The stock of ss^a used exhibits a rather constant appearance of the tarsuslike appendage of the antenna (figure 1b).

First ss^a and *d* were crossed and the F_1 flies inbred. It was possible to clearly distinguish ss^a *d* flies from the ss^a flies by the shortened leglike appendage on the antenna, thus showing the leg effect on the tarsuslike part of the antenna (figure 1c and 1d). However, the double recessive hatched about two days later than the normal flies and the number of homozygous ss^a *d* flies was actually smaller than 1/16 (243 +, 72 ss^a, 66 *d*, 13 ss^a). Most F_2 ss^a flies from these crosses exhibited a strange modification of the tarsuslike part of the antenna. The proximal half was tarsuslike, while the distal half represented an arista (figure 1e).

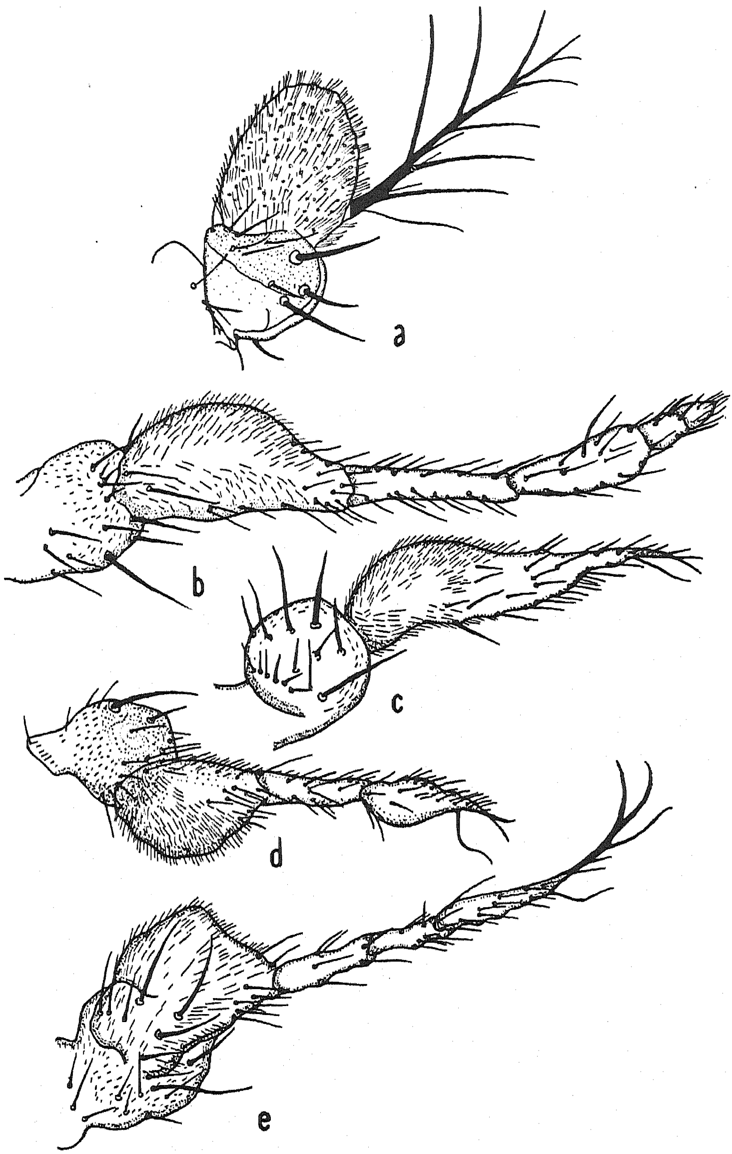
A possible explanation of this strange modification may be suggested. One might assume that a determination stream for aristapedia starts proximally in the antennal anlage of ss^a and progresses distally in a wavelike fashion. This determination process is interrupted at an early developmental point in these flies due to some as yet undetermined influence of the dachs stock. Therefore, it does not progress completely through the anlage. The distal part of the antennal anlage is thus left unchanged in these particular ss^a flies and will be determined in subsequent development by the "arista evocator." The result would be as observed, that is, an arista with a proximal leglike base.

This type could be easily selected and was kept as a true-breeding stock for several months before it was accidentally lost. It can be reproduced, however, at any time from a cross of ss^a and *d* and it deserves some extended and careful investigation.

The homozygous *tkd* ss^a flies from the F_2 of a cross between ss^a and *tkd* also showed a leg effect on the modified tarsuslike arista. These flies hatched only one day later than the normal ones and were more viable than the ss^a *d* flies of the cross previously described (312 +, 185 ss^a, 193 *tkd*, 44 ss^a *tkd*).

These combinations showed convincingly that the modified antenna of ss^a is affected by the factors which modify leg structure. It now had to be observed whether a factor which influences the normal arista would also change the modified arista of ss^a. Therefore, in the next set of experiments the mutant aristaless (*al*), which modifies the normal arista, was used. Aristaless is a recessive mutant, located in the second chromosome; it strongly reduces the arista and causes the posterior scutellars to become

erect and divergent. The count of eight bottles of F_2 flies of the cross $ss^a \times al$ gave the following results: 1052 +, 312 ss^a, 264 *al*. Only these three classes were distinguished. However, it was immediately recognized that the excessively large ss^a class probably included the "missing" ss^a *al* flies. This meant that the factor *al* showed no effect on aristapedia flies, at least as regards the altered antennae. In succeeding crosses attention was paid



FIGURES 1a–e.—a, normal antenna; b, antenna of aristapedia; c and d, antennae of "ss^a *d*" flies; e, antenna of ss^a fly from the F_2 of ss^a *d*.

to the posterior scutellars which are modified by the *al* factor. In this way the fourth class "ss^a *al*" could be distinguished. Four cultures of the F_2 ($ss^a \times al$) gave the following result: 424 normal, 105 aristaless, 115 ss^a, 50 ss^a *al*. The "ss^a *al*" flies showed no effect of *al* on the antennae of aristapedia, but its usual effect on the scutellar bristles was clearly present.

TRANSPLANTATION EXPERIMENTS

Upon the suggestion of DR. R. GOLDSCHMIDT, normal and ss^a antennal disks of approximately 400 larvae were transplanted into ss^a and normal larvae respectively. The technique developed by EPHRUSSI and BEADLE (1936) was used. Transplantations were performed between larvae of ss^a and normal at different stages of larval age from two and one-half days to pupation. The age of the larvae was determined by allowing the mother to lay eggs for 12 hours only. Donor and host were not always of same age. As a preliminary experiment normal antennal disks had been transplanted into normal larvae, in order to check whether the structure of an arista could be observed in a mature transplant. The antennal disks were usually transplanted together with the eye disk to which they are closely attached. This simplified the localization of the transplant in the mature fly. The transplant (when dissected from the adult fly) always presented the appearance of a rather disorderly mass of tissue, bristle, and chitin grown together. In about 30 percent of these control transplants, however, it was possible to locate the arista among the many bristles. The arista of such a transplant was well developed.

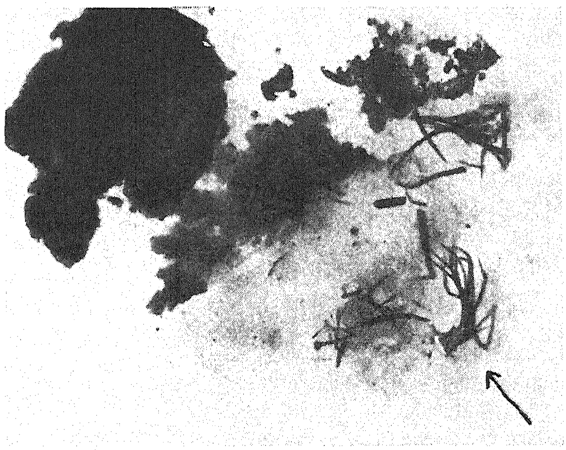
Several (nine) transplantations of normal leg disks into normal larvae were performed. No clear segmentation could be observed in the mature transplant. A spiral structure of the width of a tarsus but longer, with bristles on the inside (transplant not everted during development) was the most pronounced structure in the transplant.

After these preliminary tests were concluded, it was evident that the finding of well-developed aristae in antennal disk transplants and the spiral structure always found in leg transplants would help in analyzing the results of transplantations of disks between ss^a and normal larvae.

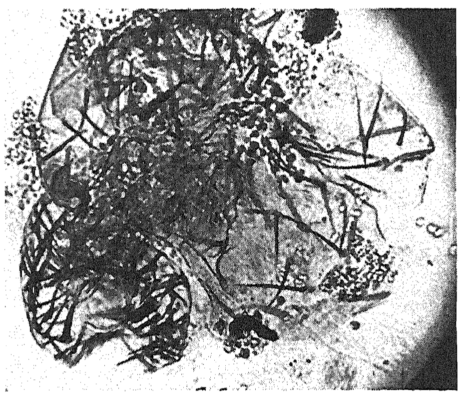
When the antennal disks of normal larvae were transplanted into ss^a larvae, at stages varying from two and one-half days of larval age to pupation, it was possible to locate a well developed arista in several cases (figure 2a). The spiral structure was never found in these transplants.

Transplants of ss^a antennal disks into normal larvae, (again at stages varying from two and one-half days to pupation) always showed the spiral structure, which is assumed to be an undeveloped tarsus (fig. 2b).

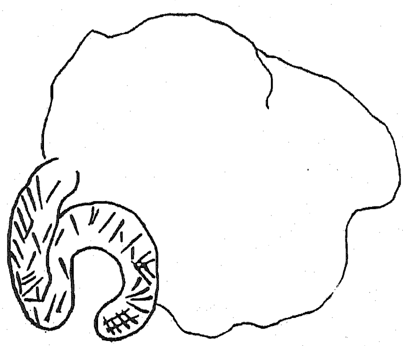
Transplantations between larvae younger than two and one-half days



2a



2b



2c

FIGURES 2a, b, c.—a, (above) a mature transplant of normal antennal disk into ss^a larva; b, (center) a mature transplant of ss^a antennal disk into normal larva. Sketch (below) shows location of spiral structure.

were not attempted, since they are extremely difficult on account of the small size of the larvae at this early stage.

DISCUSSION

These results can easily be explained on the basis of GOLDSCHMIDT'S interpretation of the development of aristapedia, and they furnish the first experimental evidence for his views.

It is to be expected that factors influencing the leg structure should also effect the leglike antenna of ss^a if the antennal disk of ss^a starts its differentiation at the same time that the leg disks begin their differentiation. This expectation has been fulfilled by the results of experiments in which the antennal structure of ss^a was observed in combinations of ss^a and different leg mutants.

On the other hand, factors influencing the structure of the arista should not be able to modify the tarsuslike antenna of ss^a. If the antennal disk of ss^a has started its differentiation at the time leg differentiation starts (at two and one-half days of larval age) it will already be far advanced in development at the time when any factors influencing the differentiation of an arista are active in the developing germ (fourth day of larval age). The already differentiated antenna of ss^a, therefore, cannot respond to such factors. This assumption has been proven in the combination of ss^a and a factor influencing the size of the arista; no effect of the aristaless factor could be observed on the antenna in homozygous "ss^a *al*" flies.

What seems at first glance to be a negative result of our transplantation experiments may be additional evidence for accelerated development of the antennal disk in ss^a. The youngest larvae used for our transplantations were somewhat older than two and one-half days, and all ss^a, therefore, had already differentiated antennal disks. The fact that ss^a antennal disks developed into tarsuslike antennae in normal larvae suggests that they may have been differentiated prior to the time of transplantation, namely at two and one-half days of larvae age. The fact that normal antennal disks developed aristae in ss^a larvae does not allow us to draw any conclusions as to the time point of determination for the arista. Determination antedates differentiation and experiments of a different nature have to be carried out in order to find the exact determination point for development of aristae. However, it has to be pointed out that these disks may be autonomous from very early stages and thence transplantation may not test their potencies. The validity of the first interpretation could be checked only if it would be possible to transplant normal antennal disks into a ss^a host prior to two and one-half days of larval age.

From these results we conclude that the ss^a factor acts by shifting the initiation of the differentiation of the antennal disks to an earlier point in

development by speeding up the development of the antennal disks. Thus it happens that the antennal disks of ss^a will be mature enough to respond to a "leg-evocator" by starting tarsus segmentation at a larval age of two and one-half days.

As GOLDSCHMIDT has already pointed out, the same explanation will be valid for other cases of homoeosis, like the mutant proboscipedia (BRIDGES and DOBZHANSKY 1933) and the mutations bithorax and tetraptera (ASTAUROFF 1929). In the latter cases the metathorax exhibits characteristics of the mesothorax; winglike structures are present instead of halteres. In the case of proboscipedia the mouth organs assume the characters of tarsi. The same simple experiments which we used to demonstrate the validity of GOLDSCHMIDT's explanation for the ss^a case, should also be performed with the mutants just mentioned.

SUMMARY

1. Homozygous combinations of dachs or thickoid with aristapedia showed that the factors influencing leg structure affected the tarsuslike part of the antenna of ss^a flies as well.

2. Homozygous combinations of ss^a with aristaless, a factor influencing the size of the arista and the position of the posterior scutellar bristles showed no effect of aristaless on the tarsuslike part of the antenna of ss^a flies, but its usual effect on the posterior scutellars.

3. Transplantations of ss^a antennal disks into normal larvae at stages from two and one-half days of larval age till pupation resulted in development of structures which were recognized as leglike.

4. Transplantations of normal antennal disks into ss^a larvae at stages from two and one-half days till pupation resulted in development of aristae.

5. These results furnish experimental confirmation of views first expressed by GOLDSCHMIDT concerning the physiology of development of the mutant aristapedia, which acts by shifting the initiation of an embryological process to an earlier point in development.

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INCOMPATIBILITY STUDIES IN COSMOS BIPINNATUS

THOMAS M. LITTLE, JEROME H. KANTOR, AND BURR A. ROBINSON, JR.
Plant Breeding Laboratories of the W. Atlee Burpee Company, Floradale Farms, Lompoc, California

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INTRODUCTION

STERILITY phenomena have been studied extensively in many genera—in Antirrhinum by BAUR (1919), FILZER (1926), and SIRKS (1927); in Capsella by SHULL (1929) and by RILEY (1932); in Linaria by CORRENS (1916) and SIRKS (1927); in Nicotiana by EAST (1915a, 1915b, 1918, 1919a, 1919b, 1919c, 1923, 1927), by EAST and PARK (1917, 1918), EAST and MANGELSDORF (1925, 1926) ANDERSON (1924), SIRKS (1927), EAST and YARNELL (1929); in Petunia by TERAO (1923) and by TERAO and U (1929); in Prunus by DORSEY (1919), SUTTON (1918), and CRANE (1925); in Verbascum by SIRKS (1917, 1926, 1927); in Veronica by LEHMAN (1919, 1922) and by FILZER (1926) and others.

But a thorough search of the available literature fails to reveal any reports on sterility in *Cosmos bipinnatus*. In fact, there seems to have been almost no study made on the sterility in any compositae. In the only Cosmos investigations recorded, MIYAKE, IMAI, and TABUCHI (1926, 1927) studied inheritance of flower color, pollen color, and floral characters but made no mention of sterility.

MATERIAL AND METHODS

In the course of breeding new varieties of Cosmos, sterility was first noted when attempts to self F_1 plants repeatedly failed to produce seed.

In 1936 a cross was made between large-flowered pink (from the variety "Sensation") and small-flowered crimson to develop a large-flowered crimson strain. The F_1 was grown in 1937 and consisted entirely of large-flowered crimson-flowered plants, indicating that crimson was dominant over pink, as had been previously recorded by MIYAKE, IMAI, and TABUCHI, and also indicated that large-flowered was dominant over small-flowered. Attempts to self individual plants in the F_1 population showed that they were all self-sterile. Therefore, four plants (well isolated from other Cosmos) were allowed to intercross at random. This produced a normal set of seed.

In 1938 a large F_2 population was grown and this population segregated for flower color and flower size. Crimson, pink, and white-flowered plants were noted, indicating that the original pink-flowered parent was heterozygous for flower color, and suggesting the existence of two factors for

color, one a basic color factor and the other a diluting factor. The segregation in flower size was approximately three large-flowered to one small-flowered. (Of 108 plants, 74 were Crimson, 17 Pink, and 17 White.)

Twenty-five large-flowered crimson plants were selected and numbered and each was individually enclosed in a cheesecloth "cage" to prevent insect pollination (all open flower-heads and seed pods were removed prior to caging). On each plant several heads were left unpollinated as checks, and it was found that none of these unpollinated flower heads produced any seed. This indicated both that all plants were self-sterile and that the cages were effective in preventing any uncontrolled pollination.

After the sterility of all the plants was established, crosses were made between all the 300 possible combinations of the 25 plants.

In Cosmos the ray florets are neutral and all of the disc florets are hermaphroditic. Anthesis of the disc florets occurs centripetally and requires about a week for completion. Emasculation was unnecessary because of their established self-sterility. Pollen was shaken from each of the male parents into an individual sterile glassine bag and in each case it was applied by camel's-hair brush to caged flower heads in which the center disc florets had just opened. At this stage, practically all of the pistils in the disc were receptive.

Within a week or ten days following pollination, its effectiveness could be determined. It was ascertained at once that some combinations were completely fertile while others were more or less completely sterile. The sterile combinations were repeated in order to ascertain whether failure to set seed was the result of inter-sterility or faulty technique in crossing. It is significant that in all cases repetition gave approximately the same negative results.

When the seed ripened, each cross was picked separately and the number of seeds recorded.

(Inasmuch as the work was done primarily to develop a large crimson strain, the 25 selections were backcrossed with a multiple recessive (small-flowered white) to determine the genetic constitution of each. No detailed sterility studies were made in this connection, however.)

RESULTS

As noted above, all 25 selections made in the F_2, as well as the F_1 population, showed complete self-incompatibility.

It was further noted that some combinations were completely cross-fertile, producing nearly a full head of seed, while others were cross-incompatible, producing no seed at all, or, at best, very few seeds.

Moreover, when a plant, A, was incompatible with each of two other plants, B and C, then B and C were also cross-incompatible with each

other. By further studies of the results, it was noted that the 25 selections fell into four intra-incompatible but inter-compatible classes as shown in table 1.

TABLE 1

*Number of seeds set per cross—arranged according to sterility grouping (Failures (under 10) repeated).**

Sel. No.	2	3	6	8	9	10	13	14	18	19	22	1	4	5	7	17	20	21	25	11	15	23	24	12	16
2		1	0	0	0	1	11	0	1	0	1	40	66	80	72	30	73	67	66	67	28	70	78	83	60
3			0	0	1	1	0	0	0	0	3	43	38	62	48	43	41	50	74	43	50	81	76	35	60
6				0	0	0	3	1	2	0	0	78	58	89	64	40	54	72	39	15	59	7	54	54	58
8					0	0	0	0	0	2	0	81	30	64	54	23	32	50	36	61	15	113	40	53	49
9						2	0	8	2	0	3	68	49	67	70	62	51	39	47	61	49	53	55	73	75
10							0	3	2	5	0	21	51	73	70	53	60	39	46	79	38	59	30	52	58
13								1	1	0	0	71	71	52	82	59	75	82	67	88	6	27	00	86	51
14									1	3	0	86	58	37	26	53	101	60	37	23	76	53	63	89	16
18										0	0	30	10	45	30	22	3	5	6	38	56	17	69	58	49
19											0	77	57	50	38	65	64	67	62	69	52	40	40	79	82
22												89	21	70	36	46	27	66	48	89	2	66	47	75	74
1													0	2	5	0	0	1	3	78	36	61	56	45	66
4														0	2	0	9	0	4	60	49	33	55	45	40
5															2	0	0	2	39	106	90	64	70	88	77
7																3	0	5	0	49	49	44	29	39	51
17																	3	0	1	106	7	51	54	81	61
20																		0	1	58	9	67	71	71	42
21																			5	82	62	50	39	72	50
25																				87	50	68	63	65	43
11																					2	4	5	75	81
15																						0	2	90	0
23																							2	92	55
24																								70	66
12																									0
16																									

* In each case where a set of less than 10 seeds was obtained, the cross was repeated. The figures represent combined set in such cases.

Average number of seeds set in compatible crosses— 55.37.

Average number of seeds set in incompatible crosses— 1.79.

Any combination which produced five seeds or less was considered as an incompatible combination. The use of the term "incompatibility" is in no way to be taken to mean the complete failure to set any seed at all, for under certain conditions, such as the end of the flowering period, a few seeds may occasionally be obtained, a manifestation of what EAST (1923) has called "pseudo-fertility." Combinations producing 15 seeds or more were considered as compatible combinations. Those producing 6 to 14 seeds were considered as questionable. On this basis, the results are tabu-

lated as in table 2, incompatibility being represented by a minus sign (−), and compatibility by a plus sign (+), with the questionable combinations symbolized by a question mark (?). According to this classification, the average number of seeds set by the incompatible combinations is 1.79, while the average number set by the compatible combinations is 55.37. This is more significant when it is taken into consideration that many of the incompatible combinations were repeated and the figure 1.79 thus represents, in many cases, more than one pollinated head of some of the combinations.

TABLE 2

Showing inter-compatibility and intra-incompatibility in Cosmos.

Sel. No.	2	3	6	8	9	10	13	14	18	19	22	1	4	5	7	17	20	21	25	11	15	23	24	12	16
2		−	−	−	−	−	?	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3			−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
6				−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	?	+	+	+
8					−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9						−	−	?	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
10							−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13								−	−	−	−	+	+	+	+	+	+	+	+	+	?	+	−	+	+
14									−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
18										−	−	+	+	+	+	+	−	−	?	+	+	+	+	+	+
19											−	+	+	+	+	+	+	+	+	+	+	+	+	+	+
22												+	+	+	+	+	+	+	+	+	−	+	+	+	+
1													−	−	−	−	−	−	−	+	+	+	+	+	+
4														−	−	−	?	−	−	+	+	+	+	+	+
5															−	−	−	−	+	+	+	+	+	+	+
7																−	−	−	−	+	+	+	+	+	+
17																	−	−	−	+	?	+	+	+	+
20																		−	−	+	?	+	+	+	+
21																			−	+	+	+	+	+	+
25																				+	+	+	+	+	+
11																					−	−	−	+	+
15																						−	−	+	−
23																							−	+	+
24																								+	+
12																									−
16																									

(−) = from none to 5 seeds per cross.
(?) = from 6 to 14 seeds per cross.
(+) = from 15 and up.

DISCUSSION AND CONCLUSION

EAST (1927) and EAST and MANGELSDORF (1925) studied the progeny of reciprocal crosses between two self-incompatible plants in Nicotiana (*N. forgetiana* × *N. alata*) and reported three incompatible classes all

inter-compatible but intra-incompatible. The progeny of a cross between members of any two classes consisted of equal proportions of two of the three, the class to which the female parent belongs not being included. Accordingly, when class X was pollinated by class Y or class Z, class X did not appear among the progeny, it consisting only of classes Y and Z in equal numbers. Three alleles for incompatibility, s_1, s_2, s_3, were assumed to explain these results. The genotypes of the three classes were represented as follows:

class X—s_1s_3
class Y—s_1s_2
class Z—s_2s_3

A plant gives stimulus only to pollen carrying genes other than its own. Therefore, pollen carrying the s_2 factor is the only type which will fertilize s_1s_3 plants (class X), and from such a pollination, half of the progenies are s_1s_2 (class Y) and half are s_2s_3 (class Z).

Further studies by EAST and YARNELL (1929) resulted in the isolation of 15 such alleles.

BAUR (1919), FILZER (1926), and SIRKS (1927) studied sterility in *Antirrhinum hispanicum* and found that the mode of inheritance was similar to that of Nicotiana. Other species in which sterility has been studied and found to be inherited in the same manner as Nicotiana are: *Capsella grandiflora* by SHULL (1929); *Linaria vulgaris* by CORRENS (1916) and SIRKS (1927); *Petunia violacea* by TERAO (1923) and TERAO and U (1929); *Verbascum phoeniceum* by SIRKS (1917, 1926, 1927); *Veronica syriaca* by LEHMAN (1919, 1922) and FILZER (1926); and others.

Our data seem to indicate that incompatibility in Cosmos is inherited in a manner similar to that in Nicotiana. Since 23 of 25 plants fell into three groups, we assume three alleles, s_1, s_2, and s_3, to be present in our population, and that the constitution of the three groups were s_1s_2, s_1s_3, and s_2s_3; and that a plant gives stimulus only to pollen carrying genes other than its own, and further that probably a fourth gene, s_4, was present in the remaining two plants, since they were compatible with the other 23 plants but not with each other.

According to the Nicotiana scheme, when the original parents of a controlled cross (not reciprocal) have three alleles, one allele in common, the genotype of the female parent does not recur in the F_1, but if the F_1 plants are allowed to intercross at random, it occurs in half the plants in the F_2, with the other two combinations each making up one-fourth of the population. Consequently, we should expect in the F_2 50 percent of the s_1s_2 (female parent) and 25 percent each of s_1s_3 and s_2s_3 plants.

In our investigation of 23 plants, 11 fell in one group, 8 in another, and

4 in the third group. On the suggested scheme, the expectations would have been 11.5 in the one group and 5.75 in each of the other two.

However, since the F_1 population was small, it is highly possible that instead of two plants each (that is, equal numbers) of s_1s_3 and s_2s_3, there were instead three s_1s_3 plants and one s_2s_3 plant. Such proportions would give in the F_2 11.5 s_1s_2, 8.625 s_2s_3, and 2.875 s_1s_3. This expectancy is more in harmony with the observed data.

The possibilities of the original parents differing by two genes is precluded by the existence of only three certain groups in the F_2. Four sterility factors would give in the F_2 six classes, a condition which did not occur.

The fourth group of two plants was assumed to carry a factor, s_4, since it was compatible with all the plants in the other three groups. The s_4 factor probably was not present in the original parents of the cross. It could easily have been introduced through contamination in the F_1, since the F_1 population was open pollinated.

When the F_2 selections were back-crossed with small-flowered white plants in unrelated lines, plants were found which were compatible with all 25 selections. This indicates the existence of at least a fifth factor for sterility.

Further investigations are being conducted to study the behavior of the various combinations in the third generation.

SUMMARY

1. *Cosmos bipinnatus* is entirely self-sterile.

2. Some plants are inter-fertile while others are inter-sterile.

3. The existence of four allelomorphs for cross-sterility has been established; and the back-cross data indicate the presence of at least an additional fifth factor.

4. Any plant gives stimulus only to pollen carrying genes other than its own, and plants of the same genotype are cross-incompatible.

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A STUDY OF THE EFFECTS OF A RANDOM GROUP OF GENES ON SHAPE OF SPERMATHECA IN DROSOPHILA MELANOGASTER

JOSEPH J. SCHWAB
University of Chicago, Chicago, Illinois

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WHETHER genes in general affect only one or a few characters of the organism, or whether most genes affect most characters is one of the fundamental problems in physiological genetics.

The consensus in the years immediately following rediscovery of Mendelian heredity was the simple concept of a one-to-one relationship between gene and character. However, instances of dual and even triple effects soon came to light; indeed, MENDEL himself (DOBZHANSKY 1927) pointed out one such effect in the pea. This led to a revision of the general view to include the possibility that a single gene might often affect multiple characters not obviously traceable to a single primary effect.

Later, it was generally recognized among Drosophila workers, that a viability lower than that of the wild type characterized most laboratory stocks containing mutations. GONZALEZ (1923) supplied quantitative data on this point, validating the general observation. However, these viability differences could not, positively, be ascribed to the single gene which caused the visible effect for which the stock was named, since there was no attempt to make the compared stocks similar for all other loci. Since then TIMOFEEFF-RESSOVSKY (1934 a, b; 1935) has tested the viability reactions of six stocks in which different single mutations were believed to be on a common genetic background. His results are positive. For three reasons, however, these positive results do not provide a basis for assuming the universality of manifold gene effects. In the first place, as HALDANE (1935) has pointed out, the commonality of genetic background is questionable since TIMOFEEFF-RESSOVSKY employed only ten backcrosses between wild type and mutant. In the second place, there is a question whether a sufficient variety of environments was tested and statistically evaluated to eliminate such environmental differences as the cause of the viability differences. Third, and most important, is the strong probability that general viability and fertility effects accompanying a single visible effect might well be the result, not of another gene effect, but of a single, fundamental action basic to both characters.

In 1927, DOBZHANSKY published the first systematic investigation of the problem, employing, not general viability or fertility effects, but a specific, quantitative character. This character was shape of the sper-

matheca in the female *Drosophila melanogaster*. Twelve mutant stocks of *melanogaster* were chosen at random, then backcrossed to wild type a number of times. After backcrossing, the spermathecal index of mutant females was compared with that of their wild-type sisters. Of the 12 stocks, ten showed significant differences in spermathecal index between mutant and wild-type individuals, a difference which DOBZHANSKY felt could be ascribed to the genes whose visible effects marked and named the stocks.

HERSH (1929) made a similar study, using facet number in a Bar-forked stock as his quantitative character. He chose the genes scute, echinus, crossveinless, cut, vermilion and garnet, and studied their effects upon facet number both singly and in combination. Taken singly, scute and echinus were found to have negligible effects; crossveinless and vermilion were strong plus modifiers; garnet and cut were strong minus modifiers. Since the experiments were carried only to the first backcross generation, there is no assurance that the modifying effects upon Bar eye were not due to closely linked genes instead of those causing the marker effects. However, these experiments have given some support to the idea that all genes may affect all characters.

Favoring a negative conclusion is the work of WARREN (1924). Examining egg size in several mutant stocks of *Drosophila melanogaster*, he found this character to be relatively constant for each stock, apparently genetic. However, his investigation indicated that egg size was a function of many genetic factors, none of which could be identified with the genes producing the visible effects which marked and named the stocks.

The work of GREEN (1931), FELDMAN (1935), CASTLE and others (1936 a, b) and of CASTLE (1938) provides interesting examples of somewhat similar studies among mammals. GREEN found, and FELDMAN and CASTLE corroborated, the fact that the gene for brown coat color in mice was accompanied by a consistent increase in body weight and body length as compared with the black mouse. A similar increase (even larger increase in tail length) accompanied the factor "dilution." Although a thoroughgoing attempt to determine whether this increase is due to the brown and dilution factors themselves, or to closely linked genes (GREEN'S interpretation) has not been feasible to date; the evidence so far leans toward the former interpretation. CASTLE (1938) has tested two other genes (albino and non-agouti) to determine whether or not they too might affect the weight and length character. For these two genes, the results were wholly negative.

There is thus a conflict of evidence at the present time. The difficulties involved in reaching definite conclusions seem to be the following: (1) the difficulty of producing two stocks isogenic except for one locus; (2) the difficulty of identifying any gene effects other than simple, additive ones,

by ordinary statistical treatment of differences; (3) the possibility of unidentified significant changes wrought by obscure environmental differences.

Because the question of the universality of manifold gene effects is both fundamental to physiological genetics and important to the genetical approach to problems of evolution, it was felt that the situation justified a reinvestigation of the problem. Shape of spermatheca in Drosophila was chosen as the character to be studied for quantitative effects as an exceptionally favorable character for this purpose and for the sake of comparison with DOBZHANSKY's previous work. The methods used are by no means original, but in a few places, especially in the statistical analysis of the data, an attempt has been made to profit by the work of others to the extent of introducing techniques designed to overcome (in part) the difficulties named in the preceding paragraph, especially the second and third.

METHODS

Metric. The method used for measurement of the spermathecae is substantially that of DOBZHANSKY. The flies chosen for measurement were removed from the bottles within four days after first emergence of adults, brought to boiling in 20 percent KOH and held there for four minutes. They were then carefully run down through 15 percent, 10 percent, and 5 percent KOH (15 minutes minimum in each) to distilled water.

The spermatheca is a dense, chitinous structure whose shape is roughly that of a derby hat without a brim. The mathematical expression of its shape is derived from two measurements, a long measurement like the diameter of a hat from front to rear, and a short measurement like the depth of crown of a hat. The ratio of these measurements provides an index of the shape of the structure. The greatest difficulty encountered is that of so adjusting the spermatheca to the axis of the microscope that the image seen is an optical section at *right angles* to the line of vision and at the plane of maximum diameter of the spermatheca (that is, the center).

For the actual measurement, not more than three (usually only two) pairs of spermathecae were placed in water on a 15 mm hollow ground slide, covered, placed under the microscope and maneuvered into the proper right angled position. Camera lucida notation was made of the long and short axes of each spermatheca, and these notations later measured with vernier calipers.

Certain precautions must be mentioned. The hollow-ground slide is necessary in order to avoid all chance of depressing the spermathecae and thereby altering the axes. It was found also that the presence of more than three pairs of spermathecae on a slide made it quite possible, by acci-

dent, to measure a member of one pair twice. Further, it was found that very small rotations of the mirror of the camera lucida about its axis, small differences in the placement of the spermathecae on the field of the microscope and small differences in position of the observing eye could make substantial differences in the measurements. For this reason, extra efforts were made for achieving consistency. First, the mirror of the camera lucida was carefully adjusted by means of a stage micrometer until it gave equal projections of a line on the stage micrometer, whether that micrometer were in the plane of tilt of the lucida mirror, or at right angles to it. Second, a pointer was placed in the microscope ocular, and every spermatheca placed with its center at this point for measurement. Third, the relative position of eye and ocular was maintained by means of contact of one part of the author's spectacle frames with a part of the camera lucida. Finally, to avoid any residual systematic error which might result from camera lucida distortion, each spermatheca was measured at whatever position relative to the tilt plane of the mirror chance happened to place it.

The consistency of this method of measurement was then tested. At intervals during routine measurements of experimental flies, a few measurements each day were duplicated on separate sides of a card. It should be noted that these were routine measures, not special ones chosen for testing accuracy. From these paired measures, indices were calculated and compared. The series of 100 differences so derived provide a basis for an estimation of error; the standard deviation of the array of differences may be interpreted as a measure of the consistency of a pair of measures and the $SD/\sqrt{2}$ as a measure of the dependability of one measure; the standard error of the mean of such an array of differences over $\sqrt{2}$ may be interpreted as a measure of the consistency of the mean of an array of indices. The values of these two parameters are:

Consistency of a single index	$.039/\sqrt{2}=.027$
Consistency of a mean of 100 indices	$.004/\sqrt{2}=.003$

Thus, it may be seen that third-place differences of means in an experiment are undependable.

Genetic methods. The method used here is a variation of the technique used by DOBZHANSKY; it consists essentially of the repeated backcrossing of each of eleven one-factor mutant stocks to a standard, wild-type isogenic stock, with measurements of the spermathecal index made before, during, and after extensive backcrossing. The details of the method differ from DOBZHANSKY, first in the use of a prepared, isogenic stock; and second in the scheme of backcrossing used.

The isogenic stock was obtained by an extension of the *ClB* principle

to cover all three major chromosome pairs. A male containing crossover-inhibiting, balanced-lethal factors on chromosomes II and III was first crossed with a *ClB* female. From the offspring, a *single* female was chosen, containing only one wild-type member of each chromosome pair. The other member of each pair carries the lethal-crossover-inhibitor combination, thus:

$$\frac{ClB}{+}\ \frac{+}{+}\ \frac{+}{+} \times \frac{+}{-}\ \frac{Cy}{Pm}\ \frac{C\ Me\ Sb\ C}{H} = \frac{ClB}{+}\ \frac{Cy}{+}\ \frac{C\ Me\ Sb\ C}{+}.$$

This female was then backcrossed to her father, yielding both male and female offspring possessing only one wild-type member of each pair of chromosomes, the other member being, as above, the crossover-inhibitor-lethal chromosome, thus:

$$\frac{ClB}{+}\ \frac{Cy}{+}\ \frac{C\ Me\ Sb\ C}{+} \times \frac{+}{-}\ \frac{Cy}{Pm}\ \frac{C\ Me\ Sb\ C}{H} = \frac{+}{-}\ \frac{Cy}{+}\ \frac{C\ Me\ Sb\ C}{+}$$

and

$$\frac{ClB}{+}\ \frac{Cy}{+}\ \frac{C\ Me\ Sb\ C}{+}.$$

These brothers and sisters were then mated *inter se*, and wild-type flies selected. Thus, a wild-type, isogenic stock is obtained in which all second and third chromosomes derive from a *single* II and *single* III chromosome from the original mother, and the X chromosomes derive from the single X of the original father. Perfect isogeneity is probable, but not certain, for there are two possible slips. In the first place, there may not be total suppression of crossing over. However, the reputation of *Cy* (which bears suppressors for both left and right limbs) is good, and so is that of the chromosome III combination. *ClB*, of course, is the best available balancer for chromosome I. In the second place, no attempt was made to inhibit crossing over in the fourth chromosome, simply because no inversion was available. However, the extreme shortness of chromosome IV itself is a crossing over inhibitor of some value.

The production of such an isogenic stock was surprisingly difficult. In the first attempt, *six* females were taken from the first mating, and each mated for 24 hours with their common father. From these six fecundations, six different brother-sister pairs were obtained and mated. In all six cases, the results were nil: each of the six attempts to produce isogeneity had rendered one or more lethal or semi-lethal genes homozygous. The *ClB* chromosome was then transferred to another stock, and another attempt made. This time, 12 independent attempts were made, each with a different pair of parents, and again, the production of stocks homozygous for

lethal or semi-lethal genes was encountered. However, from the 12, four came through, and from these four the one most fertile and viable stock was chosen.

For the extensive backcrossing of autosomal recessive mutants to this isogenic stock, the following scheme was adopted (the large letters represent wild-type alleles in the isogenic stock):

$$(1)\ a\,a \times A\,A\ =\ A\,a$$
$$(2)\ A\,a \times A\,a \qquad a\,a$$

Thus, a generation which permits insertion of haploid sets of chromosomes from the isogenic stock is followed by a generation in which crossing over between isogenic and mutant-stock chromosomes is permitted. It was planned to follow this scheme for 40 generations, thus providing 20 backcrosses interspersed with 20 crossing over matings. In all cases to follow, the notation indicating number of generations will be the number of actual backcrosses; that is, one-half of the total number of generations.

For sex-linked recessives, a similar pattern was used, thus:

(1) Isogenic female × Mutant male = Heterozygous female

(2) Heterozygous female × Isogenic male = Mutant male

Thus, *each* generation affords an opportunity for insertion of an isogenic half-genome; and each alternate generation gives free play to crossing over. It should be noted that in this case, as well as in the case of autosomal genes, only virgin females obtained by the isolation of single pupae in glass vials were used for mating.

Two new considerations now require attention. They are: (1) to what extent has the isogenic stock been substantially altered by mutations during the 36 months which the experiment has taken? (2) What average length of chromosome will remain attached on either side of the selected mutant after a given number of backcrosses?

To provide an answer to the first question, the isogenic stock, uncontaminated by any crosses, was sampled occasionally during the course of the experiment. The results for the shape index a/b are shown in table 1.

Tree characteristics of these data are relevant. First, there is no evidence of trend in the successive samples. In the light of the values to be shown later for the spermathecal index of mutant stocks, trend is known to be the best indicator of any substantial contamination of the isogenic stock. Second, successive samples show no increase in variability. This too would have resulted from an increased heterozygosity of the isogenic stock. Finally, the variability between the successive samples taken at different times can be compared with the variability between samples mated, bred, and measured at the same time. For such a comparison, a standard deviation of measures taken at different times is required. This has been com-

puted from the first five measures in the preceding table (covering the period from June, 1936, to March, 1937, inclusive), by the following method: (1) the actual variance between the five measures has been calculated by the equation, $S^2 = SV^2 - n\bar{V}^2/N - 1$. (2) Variance of means due to sampling has been calculated from the mean of the squared standard deviations of the five measures. (3) Subtraction of sampling variance from

TABLE I

Spermatheca shape in successive samples.

DATE	MEAN a/b	S.D.	C	N
6/36	2.015 ± .0174	.1754 ± .0123	8.7	102
7/36	1.886 ± .0123	.1287 ± .0087	6.8	109
9/36	1.905 ± .0180	.1797 ± .0127	9.4	100
12/36	1.962 ± .0180	.1795 ± .0127	9.1	100
3/37	1.859 ± .0105	.1049 ± .0074	5.6	100
7/37*	1.936 ± .0150	.046		10×20
10/37*	1.913 ± .0130	.034		7×20
11/37*	1.936 ± .0160	.040		6×20

* For these three cases, standard deviation as shown is based upon the variance between the means of the several bottles of flies shown under N, rather than upon the variance between flies. That the values obtained by this method are indicative of the same order of variability as in the earlier samples is shown by the following values, calculated by the old method for the July, 1937, sample:

SD = .1237 ± .0062 C = 6.4.

the actual variance gives an estimate of the true variance of the means of samples taken at different times. The values so computed are:

Actual variance	.00394
Sampling variance	−.00024
Estimate of true variance	.00370
Estimate of true SD of means	= .061

Similarly, the actual variance between means of bottles mated at the same time has been estimated from the squared deviations of the last three measures given in the preceding table. This has been corrected by deducting sampling variance as above (but based on 20 flies in each case instead of 100), yielding a corrected SD of means of bottles mated at the same time. These values are:

Actual variance	.00169
Sampling variance	−.00123
Estimate of true variance	.00046
Estimate of true SD	= .021

Application of Fisher's z test to the ratio of the variance between means from samples taken at different times, to that of samples taken at the same time indicates that the difference between these two variances is real (probability is beyond the .01 point). But taking into account the wide range of conditions under which the flies were bred from June, 1936, to March, 1937 (differences in season, climate, temperature, food formula), it is improbable that this difference indicates a substantial contamination of the isogenic stock, especially in the light of the lack of trend, and the relative stability of the variance of individual samples.

Turning now to a consideration of the efficacy of continued backcrossing as a technique for production of a common genetic background in two stocks, HALDANE and BARTLETT (1935) offer solutions to the problem for several different schemes of crossing. For the schemes used in this project, they demonstrate (for both autosomal and sex-linked recessives) that the average length of chromosome remaining on either side of the marker gene is equal to $2/M+1\ (1-.75^{M+1})$ where crossing over does not occur in one sex. The value for this expression has been approximated for certain relevant numbers of backcross generations (M, in the expression above) as shown below:

No. of backcross generations	Average length of chromosome on either side
11	16
14	13
15	13
20	9
24	8

It is obvious from the above that the assumption of one-gene differences, even after twenty backcross generations, is not tenable. Therefore, wherever, in the following pages, conclusions are drawn, such conclusions will refer, not to single genes, but to small segments of a chromosome.

Statistical methods. During the first year of the experiment, the samples chosen for measurement consisted of about 100 flies taken from one or a few bottles. The indices of such a batch were computed, and the mean, standard error and standard deviation of such a batch of 100 calculated. In May, 1937, a thoroughgoing sounding was made, which indicated that the technique was inadequate. The data disclosed the fact that bottle-to-bottle differences due to environment were so frequent that comparisons of mutant flies from one bottle with isogenic flies from another were undependable. It also made clear that the plan of the experiment would have to be modified to make possible the detection of gene actions which involved complex interaction of the genes with the environments present in

different bottles. The use of a single large sample from a single environment permits the detection only of consistent, additive effects.

To provide for these two considerations, all experiments after May, 1937, were made as follows: five or more bottles were bred, each from a mating of a single pair of flies. From the offspring within each bottle, a small, constant number (15–20) of mutant and of type flies were taken.

To question these equal, numerous, small samples statistically, the Student Method of paired comparisons was adopted as a first test. The differences between the means of the mutant and wild-type flies from each bottle were taken as the important values. A mean difference (S[d]/n) and its standard error $\left(SE_d^2 = \frac{Sd^2 - \bar{d}\,S\,d}{n(n-1)}\right)$ (where d is each difference, $\bar{d}$ the mean difference, and n the number of bottles) were computed. The ratio of the mean difference to its standard error is then tested by the usual T test.

By this means, differences of scale due to differences in bottle-environment are eliminated; but still only gene effects which result in a net difference will be detected. Very real genetic effects which involve interaction of the genes in different ways with different bottle environments go undetected. To detect this type of gene effect, and further, to provide as complete a picture as possible, a complete analysis of variance of the data of each final experiment (that is, at the conclusion of the planned number of backcrosses) was made. The methods used for this analysis will be described later, with their results.

RESULTS

The first and most direct attack upon the data consists of a T test, asking, in effect, whether or not, for each experiment, a significant difference between type and mutant flies exists at each stage during repeated backcrossing. However, the application of this test at earlier stages in each experiment is prevented. For in these earlier stages, measurements exist only for mutant flies. In order to supply the deficiency in the data, the best estimate of the mean of the isogenic flies over the whole period of the experiment has been calculated by a weighted average of all measures made. This estimate is $1.927 \pm .027$. The standard error appended here is derived from the true SD of means of samples taken at different times (.061) previously computed under "Genetic Methods." (Computation by the usual formula: $SE^2 = SD^2/N$. N, in this case, is 5.)

Whereas the mean for isogenic flies described above is a grand mean of five means, and therefore has the SE equal to $.061/\sqrt{5}$, the means of mutant flies which are to be compared with this grand mean are means of single samples. Their SE, therefore, is equal to $.061/\sqrt{1}$. From these

two standard errors of means, the standard error of their difference may be computed $SE^2_{diff} = SE_1^2 + SE_2^2$). The value of this standard error of a difference is .067, and has been so entered in table 2 of T tests. Differences carrying this standard error are differences computed from the *estimated* value of the isogenic stock. All other entries in the table are actual data or derivatives of actual data.

Two possible sources of confusion should be noted before examination of this table: (1) Whereas, for the parent, F_2 and later generations, the entry under "MEAN" is the mean index of spermathecae from homozygous mutant flies, for the F_1 generation this entry is for heterozygotes. (2) The mutants ivory and vibrissae derive from a single stock; so do the mutants wavy and cherry; hence, for each of these pairs, the measurements for the parent and first filial generations are identical.

TABLE 2

T test and other data derived from the spermathecal indices at various stages during backcrossing.

EXPERIMENT	MEAN	S.D.	N	MEAN TYPE	MEAN MUTANT	N	MEAN DIFFERENCE	T	P
Spineless									
P Gen.	1.634±.011	.1130	111				.293 ±.067		
F_1 Gen.	1.782±.013	.1358	106				.145 ±.067		
F_2 Gen.	1.793±.014	.1490	108				.134 ±.067		
5th BC*	1.758±.013	.1451	119				.169 ±.067		
10th	1.875±.016	.1277	124				.052 ±.067		
15th				1.8577	1.8976	7×15	.0399±.0337	1.18	.4 –.3
20th				1.9169	1.8791	11×15	.0377±.0202	1.87	.1 –.05
Dumpy									
P Gen.	1.476±.010	.1039	107				.551 ±.067		
F_1 Gen.	1.682±.009	.0944	107				.245 ±.067		
F_2 Gen.	1.588±.012	.1149	100				.339 ±.067		
5th BC*	1.696±.011	.1237	128				.231 ±.067		
10th	1.647±.009	.1045	135				.280 ±.067		
15th				1.8610	1.7715	5×14	.0896±.0173	5.2	.01 –.001
20th				1.9096	1.7540	11×15	.1547±.0162	9.5	.001
Brown									
P Gen.	1.733±.012	.1187	101				.194 ±.067		
F_1 Gen.	1.778±.012	.1145	100				.149 ±.067		
F_2 Gen.	1.668±.010	.0966	100				.259 ±.067		
5th BC*	1.678±.009	.1012	115				.249 ±.067		
10th	1.733±.013	.1286	100				.194 ±.067		
15th				1.8340	1.7748	8×10	.0592±.0132	4.5	.01 –.001
20th				1.9037	1.8987	10×15	.0049±.0234	.12	.9
Ebony									
P Gen.	1.945±.014	.1418	100				–.018 ±.067		
F_1 Gen.	1.849±.015	.1500	100				.078 ±.067		
F_2 Gen.	1.843±.013	.1257	100				.084 ±.067		
5th BC*	1.728±.010	.0974	100				.199 ±.067		
10th				1.9514	1.8539	5×18	.0975±.0134	7.3	.01 –.001
15th				1.9213	1.8373	7×15	.0840±.0136	6.2	.01 –.001
20th				1.9077	1.8743	10×15	.0334±.0136	2.5	.05 –.02
White									
P Gen.	1.706±.010	.0965	100				.221 ±.067		
F_1 Gen.	1.727±.009	.0939	100				.200 ±.067		
F_2 Gen.	1.727±.011	.1068	100				.200 ±.067		
4th BC*	1.738±.014	.1437	100				.189 ±.067		
7th				1.8753	1.8383	5×18	.0365±.0167	2.2	.09
11th				1.9639	1.8916	7×15	.0723±.0243	3.0	.03 –.02
15th				1.9040	1.8580	9×15	.0460±.0174	2.6	.03

TABLE 2—*Continued*

EXPERIMENT	MEAN	S.D.	N	MEAN TYPE	MEAN MUTANT	N	MEAN DIFFERENCE	T	P
Eosin									
P Gen.	1.544 ± .008	.0830	100				.383 ± .067		
F_1 Gen.	1.615 ± .009	.0850	100				.312 ± .067		
F_2 Gen.	1.617 ± .010	.0940	100				.310 ± .067		
4th BC*	1.738 ± .011	.1129	100				.189 ± .067		
7th				1.8155	1.7223	5×18	.0932 ± .0110	8.5	.000
11th				1.8556	1.8253	7×15	.0502 ± .0159	3.2	.02
15th				1.8887	1.8781	10×15	.0106 ± .0450	.2	.9 —.8
Ivory									
P Gen.	1.893 ± .010	.0979	100				.034 ± .067		
F_1 Gen.	1.929 ± .010	.1019	100				—.002 ± .067		
F_2 Gen.	1.809 ± .015	.1463	100				.118 ± .067		
4th BC*				1.9601	1.8266	5×18	.1335 ± .0309	4.3	.02 —.01
7th				1.8588	1.7629	7×15	.0974 ± .0254	3.8	.01
11th				1.8993	1.8505	7×15	.0489 ± .0242	2.0	.1
20th				1.8802	1.7989	10×15	.0813 ± .0138	5.9	.00
Vibrissae									
P Gen.	1.893 ± .010	.0979	100				.034 ± .067		
F_1 Gen.	1.929 ± .010	.1019	100				—.002 ± .067		
F_2 Gen.	1.823 ± .011	.1109	100				.104 ± .067		
4th BC*				2.0302	1.9328	5×18	.0974 ± .0308	3.2	.05 —.02
7th				1.8706	1.8102	7×15	.0603 ± .0225	2.7	.05 —.02
11th				1.7990	1.7579	7×15	.0411 ± .0112	3.7	.01
Wavy									
P Gen.	1.478 ± .010	.1005	100				.449 ± .067		
F_1 Gen.	1.603 ± .010	.0970	100				.324 ± .067		
F_2 Gen.				1.7628	1.6970	5×18	.0658 ± .0435	1.5	.2
4th BC*				1.8076	1.6930	4×18	.1146 ± .0232	4.9	.02 —.01
7th				1.9240	1.8226	5×18	.1014 ± .0239	4.2	.02 —.01
11th				1.8910	1.8407	7×15	.0504 ± .0130	3.9	.01
Cherry									
P Gen.	1.478 ± .010	.1005	100				.449 ± .067		
F_1 Gen.	1.603 ± .010	.0970	100				.324 ± .067		
F_2 Gen.				1.7292	1.7959	5×18	.0667 ± .0325	2.1	.1
4th BC*				1.8132	1.7371	5×18	.0761 ± .0336	2.3	.1 —.05
7th				1.8636	1.8001	6×15	.0635 ± .0161	3.9	.02 —.01
11th				1.8869	1.8322	6×15	.0547 ± .0228	2.4	.05
20th				1.8786	1.8931	9×15	.0144 ± .0173	.8	.4
Cardinal									
P Gen.	1.583 ± .037	.3680	100				.344 ± .067		
F_1 Gen.	1.719 ± .041	.4088	100				.208 ± .067		
F_2 Gen.	1.738 ± .014	.1426	100				.189 ± .067		
5th BC*	1.810 ± .012	.1207	100				.117 ± .067		
10th				1.9284	1.8929	5×18	—.0460 ± .0232	2.0	.2 —.1
16th				1.9254	1.8597	6×15	.0658 ± .0189	3.5	.02 —.01
Total			4261			5504			

* 5th backcross, etc.

An examination of the data in table 2 suggests three categories into which the eleven mutants fall. These are:

1. (Spineless, brown, cardinal, wavy, white, eosin, cherry.) The original parent stocks show a very large original difference in spermathecal indices. As backcrossing progresses, this difference becomes smaller, reaching a point of near insignificance in all but cardinal and wavy. The progressive decrease in the difference suggests that a large number of loci in these

stocks were occupied by genes which altered the spermathecal index, and further, that an effective majority of the modifiers affected the index in one direction only (that is, made it smaller than the index of the wild type) a direction which will be referred to in the future as positive.

2. (Ebony, vibrissae, and ivory.) In these three stocks, the original difference between mutant and wild-type indices is inconsequential. But following continued backcrossing, a significant increment is built up and maintained by ivory, but nearly lost in the other two stocks. The suggestion here is that again many loci modify the spermathecal index, but in such a manner that plus and minus modifiers nearly neutralize each other. During early stages of backcrossing more minus than plus modifiers are sloughed off (more plus than minus modifiers closely linked to ebony and vibrissae?) producing a plus difference in spermathecal index. During further backcrossing, plus modifiers are removed from ebony and vibrissae, bringing the difference between mutant and wild-type indices back to a nearly insignificant value.

3. (Dumpy.) Like category 1, above, the original stocks show a large difference. But unlike the mutants in category 1, a *large* significant part of the original difference remains after extensive backcrossing. The situation here suggests that, although many other loci may have contributed to the original difference between mutant and wildtype, a large part of that difference is due to one or more loci within ± 9.5 crossover units of dumpy, perhaps is due to dumpy itself.

It is also of interest to note the changes which take place in the shape index between the P, F_1 and F_2 generations. The F_1 generation is, of course, not only heterozygous for the marker gene, but also for all the other loci (with respect to the alleles introduced from the isogenic stock). Yet, the F_1 in most cases (and especially in the wavy-cherry stock) displays little tendency to move toward the index of the isogenic stock. The implication is that the shape index is determined by many factors, among which the marker gene is of no greater consequence than those whose presence is not indicated by external effects. This tentative conclusion is supported by the fact that in some of the stocks, F_2 flies, homozygous for the marker gene, do not differ markedly from the F_1 flies which are heterozygous for this gene. This is true of spineless, ebony, white, and eosin.

Confining attention to the final measurement alone, the following summary appears to be justified:

MUTANT STOCK	THE FINAL NET DIFFERENCE IS:
dumpy	significant
wavy	significant
cardinal	significant

MUTANT STOCK	THE FINAL NET DIFFERENCE IS:
vibrissae	significant
ivory	significant
ebony	equivocal
white	equivocal
spineless	equivocal
brown	not significant
eosin	not significant
cherry	not significant

Remembering that the terms, *significant, equivocal,* and *not significant* in the above list refer only to net differences due to additive, consistent effects of the genes, we turn now to the statistical method for detection of both simple additive and complex, inconsistent interaction effects.

To describe the methods of computation used in the analysis of variance, we shall take a hypothetical experiment involving ten bottles of flies. From each of the ten bottles, the mean spermathecal index of 15 mutant, and 15 wild-type flies is determined. These data are arranged in a two-row table, allotting a column for each bottle. For each bottle, the mean of the wild-type sample is entered in the top row; the mean of the mutant sample is entered in the bottom row, thus:

ARRANGEMENT OF DATA FOR ANALYSIS OF VARIANCE

t′	t′	t′	t′	t′	t′	t′	t′	t′	t′	T′	$\overline{\text{V}}$
t	t	t	t	t	t	t	t	t	t	T	
B	B	B	B	B	B	B	B	B	B		
d	d	d	d	d	d	d	d	d	d		

Each t′ represents the mean of 15 wild-type indices; each t represents the mean of 15 mutant indices from the same bottle. T and T′ represent the means of their respective rows: B represents the mean of its column (bottle mean); and $\overline{\text{V}}$ represents the grand mean. Each d represents the difference between type and mutant means for its bottle.

From these data, variance among flies will be estimated in seven ways, whose intercomparisons will provide us with the desired information.

The first of these, to be called the *between type* variance (that is, an estimate of variance among flies, assuming no real difference between types), is computed from T, T′, and $\overline{\text{V}}$, by the following equation (N = degrees of freedom, and K = weighting factor):

$$\text{Between type variance} = \text{KS } (\text{T} - \overline{\text{V}})^2/\text{N}.$$

The values of N and K will be given in the summarizing table. (They differ in the different formulae.)

The second estimate (to be called the *between bottle* variance) is computed from the B's and $\bar{V}$, thus:

$$\text{Between bottle variance} = KS\ (B-\bar{V})^2/N.$$

Description of the third (to be called *interaction* variance) will be deferred momentarily for a description of the fourth (to be called *sub-total* variance). This sub-total variance is computed from t, t′, and $\bar{V}$, thus:

$$\text{Sub-total variance} = KS\ (t-\bar{V})^2/N.$$

The third (interaction) variance, mentioned above, is computed by summing the sums of squares of *between type* and *between bottle* variance, and subtracting this sum from the sum of squares of the sub-total variance; that is:

$$\text{Interaction variance} = \frac{KS(t - \bar{V})^2 - (KS(T - \bar{V})^2 + KS(B - \bar{V})^2)}{N}.$$

The fifth variance (to be known as *individual* variance) is computed not from our tabulated means, but from the values of each index of each individual fly, and the mean (t, or t′) of the group to which it belongs, that is (using i to represent the index of individual flies):

$$\text{Individual variance} = KS(i-t)^2/N.$$

The sixth variance (to be called the *total* variance) is computed from the values of individual flies and the grand mean, thus:

$$\text{Total variance} = KS(i-\bar{V})^2/N.$$

The seventh variance (to be known only as variance 1+3) is computed simply from the sum of the sums of squares of *between type* and *interaction* variance, thus:

$$1 + 3 \text{ variance} = \frac{KS(T - \bar{V})^2 + \text{interaction sum of squares}}{N}.$$

All of this information, plus the actual values of K and N for each estimate of variance is summarized in table 3.

The meaning of each of these estimates of variance is as follows:

The total sum of squares $S^{2mn}(i-\bar{V})^2$ with 2 mn−1 degrees of freedom can be analyzed into two necessarily independent parts: (No. 5), flies from bottle-type means, $S^nS^{2m}(i-t)^2$, with 2(m−1) degrees of freedom, and (No. 4) bottle-type means from the grand mean, m $S^{2n}(t-\bar{V})^2$, with 2n−1 degrees of freedom. (S^x = summation x times.)

The latter, sum of squares of bottle-type means from the grand mean,

can in turn be analyzed into two necessarily independent parts: (No. 2), bottle means from the grand mean, $2m\ S^n(B-\bar{V})^2$ with $n-1$ degrees of freedom, and (No. 7) residual variance, $m\ S^{2n}(t-B)^2$ with n degrees of freedom.

A still further analysis of this residual, No. 7, can be made into two necessarily independent parts. These are No. 1, of type means from the grand mean, $mn\ S^2(T-\bar{V})^2$ with one degree of freedom, and No. 3, interaction of type and bottle deviation, $m\ S^{2n}((t-B)-(T-\bar{V}))^2$ with $n-1$ degrees of freedom. (Note that $(T-\bar{V})$ is the mean of $(t-B)$.)

TABLE 3

Summary of analysis of variance.

NO.	NAME	SUM OF SQUARES	VALUE OF K*	DEGREES OF FREEDOM
1	Betw. Type	$KS(T-\bar{V})^2$	$n\times m$	1
2	Between Bottles	$KS(B-\bar{V})^2$	2 m.	9
3	Interaction	#(4) below, minus the sum of Nos. (1) & (2) above.	—	9
4	Sub-total	$KS(t-\bar{V})^2$	m	19
5	Individual	$KSS(i-t)^2$	1	280
6	Total	$KS(i-\bar{V})^2$	1	299
7	1+3	The sum of Nos. (1) and (3) above.	—	10

* n = No. of Bottles. m = No. flies per type-sample (15 in all cases).

The ones of greatest interest for the present problem are Nos. 1, 3, 7, and 5. The latter is merely the individual variance of flies from their bottle-type mean. Some light on the nature of the others is obtained by expressing them in similar terms, namely the difference d between type and mutant means within a bottle, and $\bar{d}$, the mean of such differences for an experiment. The expressions are:

$$\text{(No. 1)} \qquad \text{Between type } mn\overset{2}{S}(T-\bar{V})^2 = mn\frac{\bar{d}^2}{2}$$

$$\text{(No. 3) Interaction } \frac{m\overset{2n}{S}((t-B)-(T-\bar{V}))^2}{n-1} = \frac{m\,S(d-\bar{d})^2}{2(n-1)}$$

$$\text{(No. 7)} \qquad \text{Residual } \frac{m\,S(t-B)^2}{n} = \frac{m\,S\,d^2}{2n}.$$

For each final measurement on each mutant used, table 4 summarizes the numerical values of these estimates of the variance.

We may now test these data for whatever significant information they may yield. The principle of the test is as follows: each estimate of variance

(as, for instance, the estimate based upon type means, No. 1) may be considered as a way of determining the variance of flies; if there is no real difference between types, the type means estimate should not differ sigficantly from the estimate based directly upon the individual fly measurements, No. 5. We test the two estimates to determine the probability that the difference from equality may occur by chance. For such a test, Fisher's z (1932) or Snedecor's F (1934) (which is derived from Fisher's z) test may be employed. For convenience, the latter has been chosen.

TABLE 4

Analysis of variance—numerical values.

(Values in parentheses are degrees of freedom.)

MUTANT	ESTIMATE DERIVED FROM			
	BETWEEN TYPES (1)	INTERACTION (3)	WITHIN BOTTLE DIFFS. (7)	INDIVIDUAL DIFFERENCES (5)
spineless	.1191 (1)	.0332 (10)	.0410 (11)	.0137 (308)
dumpy	2.1523 (1)	.0182 (10)	.2122 (11)	.0142 (308)
brown	.0005 (1)	.0361 (9)	.0326 (10)	.0144 (280)
ebony	.0835 (1)	.0139 (9)	.0209 (10)	.0103 (280)
white	.1431 (1)	.0205 (8)	.0341 (9)	.0147 (252)
eosin	.0099 (1)	.0208 (9)	.0197 (10)	.0110 (280)
ivory	.4948 (1)	.0143 (9)	.0624 (10)	.0153 (280)
cherry	.0142 (1)	.0201 (8)	.0195 (9)	.0109 (252)
wavy	.1332 (1)	.0089 (6)	.0266 (7)	.0167 (196)
cardinal	.1948 (1)	.0160 (5)	.0458 (6)	.0174 (168)
vibrissae	.0885 (1)	.0066 (6)	.0183 (7)	.0130 (196)

The test consists simply of determining the following ratio of variances: 1/3, 1/5, 3/5, and 7/5, and finding the probability that, for the appropriate number of degrees of freedom upon which each estimate was based, a random deviation would produce a ratio as great or greater than the one found.

If the variance based upon one degree of freedom (No. 1) is found to deviate from the estimate based upon individual fly measurements to an extent that would occur only once in a hundred trials, we conclude that our original assumption was false; that is, that contrary to our assumption, there *is* a real difference between types. Similarly, we may test estimates (3) and (7) against the estimate from individual differences (No. 5). We may also make a further comparison; of estimate (1) to estimate (3). The meaning of this comparison may be deduced from the values in terms of d of estimate (1) and estimate (3), derived in the preceding paragraphs. Estimate (1) was found to be equal to $\frac{1}{2}$n m $\bar{d}^2$. Estimate (3) was found

to be equal to $\frac{1}{2}m\, S(d-\bar{d})^2/n-1$. The ratio of these two is: $n-1 \dfrac{n\bar{d}^2}{S(d-\bar{d})^2}$.

In short, test of estimate (1) against estimate (3) is identical with the Student's T test previously used. This provides an interesting link between the simpler test and the present ones.

The significance or non-significance of each of these four tests (that is, 1/5, 1/3, 3/5, and 7/5) may, to some extent be interpreted separately. If the ratio 1/5 be found to be significantly large, it may reasonably be concluded that one or more bottles of flies have contributed values of the shape index for mutant and wild-type flies of a difference greater than would be expected to occur by chance from the variability of flies within a genetic type within a bottle. Similarly, if the ratio 1/3 be significantly large, a *consistent* difference between types within bottles greater than would be expected by chance from fly variability may be concluded; that is, a regular, additive effect of the genes involved is demonstrated. If the ratio 3/5 be significant, it may be concluded that a real genetic effect involving interaction with bottle environments is present. The ratio, 7/5, is the best single test for the existence of any residual variability within bottles, but does not distinguish between additive and non-additive effects (interaction) of the mutation.

For such individual interpretations of separate ratios, a table (table 5) of the values of the ratios, and the probability of a ratio as large or larger occurring by chance is given. The F column is the actual ratio. Because of the fact that the tables of the distribution of F which are available are limited in their scope, actual values are not given under P. Instead, a plus sign is used to indicate a probability of .01 or smaller; an interrogation mark indicates a probability between .05 and .01; and a zero indicates a probability greater than .05. It should be noted that for ratios less than one, the table of F (table 5) has been entered for the appropriate number of degrees of freedom with the reciprocals of these ratios. None has been found to be improbably smaller than unity.

As has been stated, some information can be derived from inspection separately of the significance of the ratios. However, it is the entire constellation of the significances which, taken as a whole, give the best picture of the kind (if any) of genetic determination of spermatheca shape which is operating in each mutant stock. Because the permutations of four things, where each may have any one of three values (+, ?, or o), is unwieldy, a graphic rather than a tabular representation has been chosen.

The graphic representation in figure 1 has been suggested by PROFESSOR SEWALL WRIGHT. Taking the values of ratio C as abscissa and ratio B as ordinate, the results for any experiment can be shown relative to the

lines which indicate complete randomness ($F = 1$), and deviations from randomness indicated by lines indicating the values of F when the probabilities are .99, .95, .05, and .01.

Since ratio A can be shown to be equal to ratio B/ratio C, the equiprobable lines for this ratio can be indicated as radiating from the lower left-hand corner. Equiprobable lines for ratio D (which is equivalent to ratio $B+(n-1)$ ratio C/N) can be shown cutting across these from lower right to upper left. Thus, a single point can indicate the significance of the results of an experiment relative to all four ratios.

TABLE 5

F test of estimates of variance.

MUTANT	RATIO A $\frac{1}{3}$		RATIO B $\frac{1}{5}$		RATIO C $\frac{3}{5}$		RATIO D 7/5	
	F	P	F	P	F	P	F	P
Spineless	3.6	o	8.7	+	2.4	+	3.0	+
Dumpy	118.8	+	151.6	+	1.3	o	14.9	+
Brown	.01	o	.03	o	2.5	+	2.3	?
Ebony	6.0	?	8.1	+	1.3	o	2.0	?
White	7.0	?	9.7	+	1.4	o	2.3	?
Eosin	.5	o	.9	o	1.9	o	1.8	o
Ivory	34.6	+	32.4	+	.9	o	4.1	+
Cherry	.7	o	1.3	o	1.8	o	1.8	o
Wavy	15.0	+	8.0	+	.5	o	1.6	o
Cardinal	12.2	?	11.2	+	.9	o	2.6	?
Vibrissae	13.4	+	6.8	+	.5	o	1.4	o

As already noted, the location of this point relative to D gives the best single indication of *any* effect of the mutation, but does not distinguish between additive and non-additive effects. (Indeed, as may be seen from the diagram, high significance in *one* of these may not be revealed in D.) The best indication of an additive effect is given by ratio A (equivalent to Student's T test), while location on the C axis is the best indicator of the presence of interaction. The B scale is principally valuable for confirmation of the conclusion from the A scale in the region where C is improbably small (extreme left).

Two special features of the specific diagram should be noted. First, because of reproduction difficulties it was not found feasible to place the .99 and .95 lines for ratios A and B, since they fall too near the edge of the graph. Second, in a strictly accurate presentation, separate diagrams of this kind for each number of bottles used in an experiment should be utilized. However, the present diagram (laid off for $n = 10$) would be negligibly different for $n = 11$ and $n = 9$. For those experiments containing six

or seven bottles, the results, by chance, have fitted satisfactorily into this diagram. Third, the B value for dumpy is greater than shown.

SUMMARY AND CONCLUSIONS

1. Seven segments of chromosomes show direct, additive effects upon

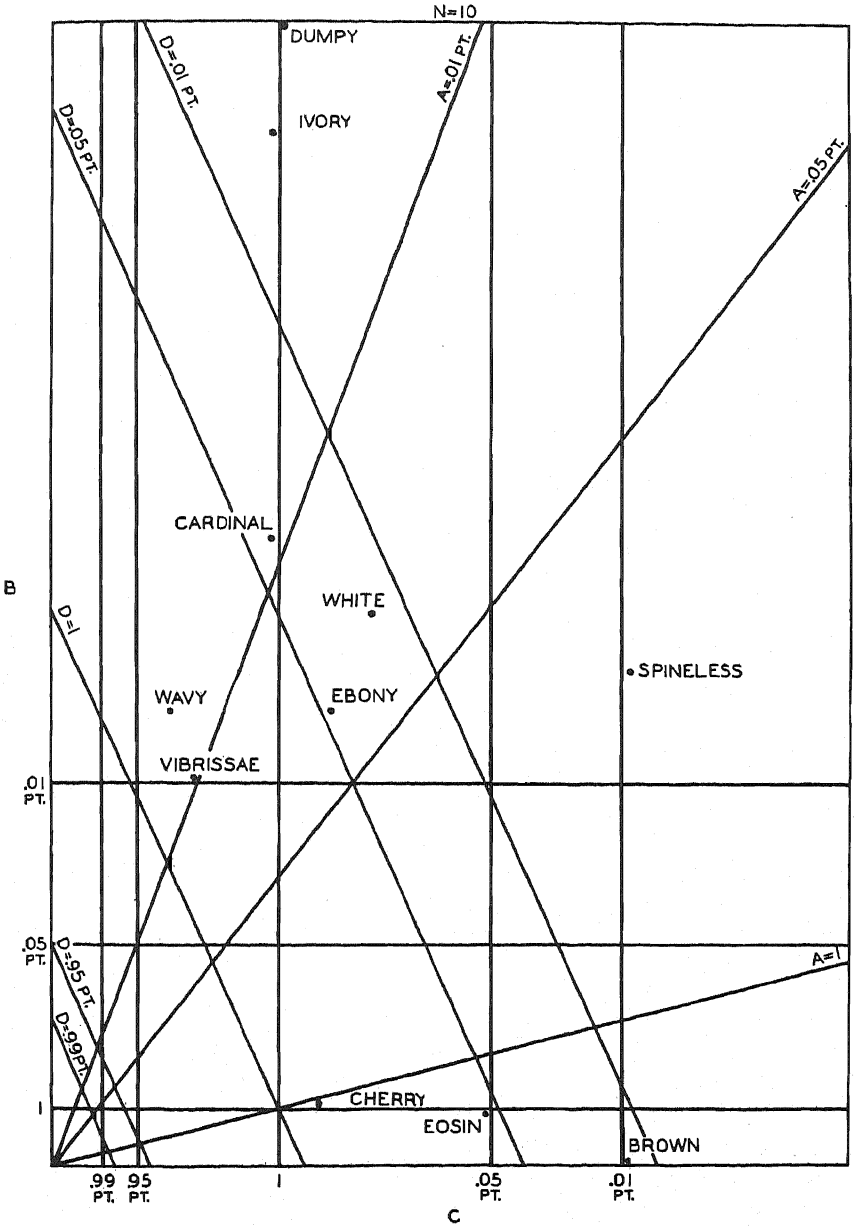

FIGURE 1.—Graphic representation of F tests of estimates of variance.

spermatheca shape. These are: wavy, vibrissae, cardinal, dumpy, white, ivory and ebony. Of these, only dumpy and ivory show a mean difference from the isogenic index of relatively great size. All the others are small.

2. Only one segment of chromosome (brown) shows a complete absence of an additive effect coupled with presence of an interaction effect.

3. One chromosome segment (spineless) shows a strong interaction effect upon spermatheca shape coupled with some evidence of direct, additive effect.

4. Two segments of chromosome (eosin and cherry) show a complete absence of any genetic effect whatsoever.

These results differ in kind from the results achieved by DOBZHANSKY. In twelve stocks tested by him, ten were found to have strong, additive effects. The other two were negative. (These results include, however, several stocks carried through only a few backcross generations.) The results presented here, on the other hand, show only two cases of a *strong* purely additive effect although there are five additional cases of small purely additive effects; and of the remaining four, two show additive effects in stocks also showing interaction effects.

Despite the difference in kind of results, the conclusions based on the data presented here, support, in general, the conclusions of DOBZHANSKY. His paper, written before the appearance of HALDANE'S paper on the value of backcrossing as a producer of isogeneity, assumed apparently that after 20 to 30 generations of backcrossing or inbreeding, residual differences between mutant and wild-type flies could be attributed to the one-gene difference. On that basis, DOBZHANSKY'S tentative conclusion was that there was a high probability that of 12 genes with external, visible effects upon the fly, ten also affected shape of spermatheca. The evidence here presented provides a basis for concluding that, of eleven chromosome segments (containing a gene with external, visible effects) nine are found to contain genes which affect spermatheca shape. Those genes may be one or many; include or exclude the gene with the external, visible effect.

It remains true, nevertheless, that of 11 chromosome segments chosen purely for convenience, nine were found to affect an organ chosen purely for convenience. This is strong evidence for the proposition that many structures are affected by a multiplicity of genes. But for the converse of this proposition, namely that many genes affect a multiplicity of characters, these data offer no evidence. For it cannot be assumed that these ten segments achieve their effect upon spermatheca shape by virtue of the contained gene which affects an external character.

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I wish to take this opportunity to thank DR. SEWALL WRIGHT for the

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HANDEDNESS, WITH SPECIAL REFERENCE TO TWINS

D. C. RIFE
Ohio State University, Columbus, Ohio

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INTRODUCTION

WHILE the occurrence of right-and left-handedness does not appear to conform to any simple Mendelian formula, the familial incidence of left-handedness rather definitely indicates a genetic basis. Children are more likely to be left-handed if one parent is left-handed, than if both are right-handed (CHAMBERLAIN 1928), and in families where *both* parents are left-handed about 50 percent of the children are also left-handed.

Students of twins generally agree (NEWMAN 1928, LAUTERBACH 1925, WILSON and JONES 1934) in finding a higher percentage of left-handedness in both monozygotic and dizygotic twins than in single born populations. This increase is due principally to the frequent occurrence of intra-pair differences in handedness, rather than to any excess of pairs in which both members are left-handed. Both types of twins differ from the single born in that the conditions *in utero* are necessarily changed. But if the position *in utero* is responsible for the differences in the expression of handedness in certain pairs of twins, why does it not affect *all* twin pairs similarly?

NEWMAN (1928, 1937) is of the opinion that in monozygotic twins, embryonic division occurs near or during gastrulation. As the axis of the embryo and bilateral asymmetry are established during gastrulation, he assumes that monozygotic twins which do not separate until after gastrulation will exhibit intra-pair differences in handedness, whereas those separating before gastrulation will be of the same handedness. He also attributes intra-pair variations in other bilateral asymmetrical traits, such as dermatoglyphics, direction of head hair whorl and dentition to the same cause. But if this is the correct explanation, there should be a definite stage in embryonic development for the establishment of each trait showing bilateral asymmetry, beyond which separation of the embryo should result in intra-pair variation, but if separation should occur at some previous time the members of the pair would be alike. Thus we should expect definite combinations of intra-pair trait reversals in monozygotic twins. For example, we might expect to find some twins showing no intra-pair differences in either handedness or direction of hair whorl, some to show reversals in handedness and the same direction of head hair whorl (assuming handedness normally to be established first), some to show differences in both traits, but none to show reversals in hair whorl and the same handedness. Actually, however, we find all possible combinations in re-

spect not only to handedness and hair whorl, but also to any other traits involving bilateral asymmetries. We might logically expect to find *situs inversus viscerum* in one member of monozygotic twin pairs in a high percentage of cases, but actually such cases are extremely rare. Furthermore, Newman's hypothesis completely fails to account for the excess of left-handedness in dizygotic twins.

LAUTERBACH (1925) offers an alternative explanation in which he states "the causes which operate to produce twins also operate to produce left-handed individuals." Considering the quite different modes of origin of the two types of twins, such a hypothesis seems questionable. If true, however, we might expect a higher percentage of left-handers in the non-twin members of the families of twins, than in non-twin families. Such a comparison is included in this paper.

The writer (1938) has observed an apparently high frequency of left-handers among the relatives of twins showing intra-pair variations in handedness, as contrasted with a low frequency of left-handed relatives of twins where both members are right-handed. These observations have suggested a different hypothesis, which could apply to both monozygotic and dizygotic twins. Handedness is assumed to be a quantitative trait, and those individuals who are genotypically intermediate may easily be shifted one way or another in the determination of manual preference. Unusual position *in utero* is one circumstance which may finally condition the handedness of such individuals. The handedness of individuals genotypically strongly right-handed or strongly left-handed, would not be affected by circumstances *in utero*. Thus monozygotic twins showing intra-pair reversals in handedness are assumedly genotypically intermediate in handedness, and the unusual position *in utero* results in one becoming left-handed and the other right-handed. Members of fraternal twin pairs are, of course, of different genotypes, but here, too, the unusual position *in utero* may be sufficient to condition the handedness of genotypically intermediate individuals. A similar explanation for the increase of left-handedness in twins was given by VERSCHUER (1931, 1932).

If the above hypothesis is correct, we should expect to find a higher incidence of left-handedness among the relatives of pairs containing one right-handed and one left-handed member than among the relatives of pairs where both members are right-handed. In this paper we present an analysis of data of our own, as well as that of various other investigators, on the incidence of left-handedness in both twins and the single born.

THE DIAGNOSIS OF HANDEDNESS

There is no general agreement among students of handedness as to just what constitutes right-handedness or left-handedness. The fact that an

association exists between the kicking foot, the dominant eye and the preferred hand has led some investigators (LECHE 1933) to include the kicking foot and the dominant eye among the criteria of handedness. DOWNEY (1927) included both unimanual and bimanual operations in her analyses of handedness. JONES (1918) classified people as to handedness on the basis of arm measurements. NEWMAN (1937) used electrical tapping tests as his criteria for the handedness of twins. Novel tests for handedness, such as blindfolding a person and seeing which way they turn when walking have also been suggested. OJEMANN (1930) in an analysis of artificial tests of handedness such as tapping tests, has shown them to be unreliable. Testimony as to preferred hand, and actual performance appear to be the most reliable tests of handedness. It is hazardous, however, to classify handedness on the performance of only one or two types of tests as has too frequently been done. Writing, for example, is a poor criterion when used alone, in determining the incidence of left-handedness, as so many left-handers have been trained to write with their right hands. When used with other criteria, it is of considerable value, as only rarely do we find right-handers writing with their left hands. Throwing is an excellent criterion for males, but unreliable for females, whereas the hand used for holding a needle is a good criterion for females, and a poor one for males.

In our own classification of handedness, we refer to the hand preferred in the performance of unimanual operations. Our data on an individual's handedness includes his testimony as to whether he considers himself right- or left-handed, or ambidextrous, and the preferred hand for each of the following:[1]

1. Throwing
2. Bowling
3. Marbles
4. Knife
5. Spoon
6. Hammer
7. Saw
8. Sewing
9. Writing
10. Scissors

We have arbitrarily grouped those tested into two classes, right-handers and left-handers. Right-handers include only those who use the right hand for all ten operations, and left-handers those who use the left hand or either hand with equal ease in one or more of the operations.

THE INCIDENCE OF LEFT-HANDEDNESS IN THE GENERAL POPULATION

We recently conducted a survey of the handedness of 687 families. All were families of whom one or more members were students at Ohio State University, taking some course in elementary zoology. Questionnaires

[1] These criteria were suggested by PROF. J. M. RIFE, of Muskingum College.

were prepared asking about the handedness of each member of the family in respect to all items included in our criteria. The students were carefully instructed in the classroom as to how to take the data, and were cautioned not to answer any items concerning which they could not obtain first-hand information. As the questionnaires were given out just before Thanksgiving vacation, the students, with only a few exceptions, had

TABLE 1

The familial occurrence of right- and left-handedness.

TYPE OF CHILDREN	R♂×R♀ (620 MATINGS)	R♂×L♀ (30 MATINGS)	L♂×R♀ (32 MATINGS)	L♂×L♀ (5 MATINGS)
R♂	1084	33	40	2
R♀	758	26	41	3
R♂+♀	1842	59	81	5
L♂	105	6	9	3
L♀	46	10	9	3
L♂+♀	151	16	18	6

opportunities to directly contact the members of their immediate families. Table 1 summarizes the raw data. The excess of males is due to the fact that the great majority of elementary zoology students are males. In a total population of 3542 we find that 263, or 7.45 percent are left-handed. Table 2 shows a close agreement between our findings and those of other investigators.

TABLE 2

Frequencies of left-handedness in the general population.

	NO. OF INDIVIDUALS TESTED	%L
JONES and WILSON	521	6.5
NEALL	800	6.5
QUINAN	1000	7.6
RIFE	3542	7.45

Of the 263 left-handers, 100 are left-handed in all ten operations, and 33 are left-handed in all operations except writing. Twenty-eight are left-handed in only one operation, of whom thirteen are left-handed in throwing, and one is left-handed in writing. No individuals are right-handed only in throwing. Left-handed children occur in families where both parents are right-handed, and five out of a total of eleven children are right-handed in families where both parents are left-handed. These figures indicate the quantitative nature of handedness and clearly show that its inheritance cannot be explained solely on the basis of a single pair of factors.

The fact that left-handed children occur more frequently in families

where one or both parents are left-handed is strikingly shown in the 2×2 table (table 3). Here we find $\chi^2 = 41.7$. As any value of χ^2 beyond 3.841 is considered to be significant, in an analysis involving one degree of freedom, no further comment seems necessary to indicate the tremendous significance of the above differences. It is of interest to remark,

TABLE 3

Two-by-two table showing the incidence of right- and left-handedness among offspring where both parents are right-handed, as contrasted with offspring where one or both parents are left-handed.

	TYPE OF MATING		
	R×R	R×L & L×L	TOTALS
R children	1842	145	1987
L children	151	40	191
Totals	1993	185	2178

$\chi^2 = 41.7$.

however, that our data include a family of five, of which both parents and all three children are left-handed. The children perform all diagnostic operations left-handed, and the parents are left-handed in all except writing. As only about 50 percent of those classed by us as left-handers are as completely left-handed as members of this family, the frequency of the occurrence of such families, if handedness were purely a matter of chance, should be approximately one in a million families of five ($.04^5$).

HANDEDNESS OF TWINS

Numerous investigators have collected data on the incidence of left-handedness in twins. Unfortunately, there has been little uniformity as to criteria used, and some investigators even fail to state any. The writer has obtained data on the handedness of 223 monozygotic and 146 dizygotic pairs of twins. Each pair was tested by the writer and his assistants.

TABLE 4

The frequencies of R-R, R-L and L-L pairs in monozygotic and dizygotic twins.

	MONOZYGOTIC								DIZYGOTIC							
	R-R		R-L		L-L				R-R		R-L		L-L			
	NO.	%	NO.	%	NO.	%	TOTAL	% OF L INDIVIDUALS	NO.	%	NO.	%	NO.	%	TOTAL	% OF L INDIVIDUALS
Newman	30	60	17	34	3	6	50	23	39	78	11	22	0	0	50	11
Wilson & Jones	56	80	13	18.6	1	1.4	70	10.7	97	88.9	24	19.5	2	1.6	123	11.4
Rife	176	79.5	41	18.3	6	2.2	223	11.35	104	71.3	39	26.7	3	2	146	15.35
Totals	262	76.0	71	20.7	10	3.4	343	14.0	240	75.3	74	23.2	5	1.5	319	13.1

Table 4 is a summary of the data obtained by three American investigators. WILSON and JONES (1934) used throwing and writing as their criteria for handedness. NEWMAN (1937) used tapping tests and testimony as diagnostic criteria. WILSON and JONES' data on monozygotic twins agree closely with our own whereas NEWMAN finds a significantly greater frequency of left-handers in this group. Among dizygotic twins we encountered a higher frequency of left-handedness than did either NEWMAN, or WILSON and JONES. None of the differences in this latter group, however, are statistically significant. All three investigators agree in finding significantly higher percentages of left-handedness in both types of twins than the 7.45 percent obtained in our group of single born individuals.

Table 5 shows the relative frequencies of R-R, R-L and L-L sib pairs in our group of single born individuals, as contrasted with similar groupings for both types of twins. It is apparent that the higher incidence of left-handedness in twins is due principally to the relatively high frequencies of R-L pairs.

TABLE 5

Comparative frequencies of R-R, R-L and L-L pairs in monozygotic twins, dizygotic twins and paired sibs.

	R-R		R-L		L-L	
	No.	%	No.	%	No.	%
Monozygotic	262	76	71	20.7	10	3.4
Dizygotic	240	75.3	74	23.2	5	1.5
Sibs	3067	85.6	475	13.2	41	1.2

THE INCIDENCE OF LEFT-HANDEDNESS IN THE IMMEDIATE FAMILIES OF TWINS

Table 6 summarizes in two-by-two tables our data in regard to the handedness of the immediate relatives of twins. By immediate relatives we refer to parents and sibs. For the identical twins $\chi^2=12.8$ and for the fraternal $\chi^2=18.1+$. Such values show conclusively that left-handedness occurs more frequently among the immediate relatives of R-L twins, than among the relatives of R-R twins. This is in harmony with what we might expect, if R-L twins are genotypically intermediate in handedness.

Table 7 shows data in two-by-two tables pertaining to the relative frequencies of left-handedness of the single-born in families containing twins, and those containing no twins. Table 7A shows that the incidence of left-handedness is significantly less in the immediate relatives of monozygotic twins, than in non-twin families. This is just the reverse of what we might expect if the same factors are responsible for left-handedness and

twinning, as assumed by LAUTERBACH (1925). We are at a loss, however, to explain why left-handedness should occur with significantly *lower* frequency among the relatives of monozygotic twins. Table 7C shows no significant differences between non-twin families and the families of dizygotic twins in regard to the incidence of left-handedness. Also, when the twins are included, as shown in tables 7B and D, there is no significant

TABLE 6

Two-by-two tables showing the comparative frequencies of left-handed relatives for R-R and R-L twins.

MONOZYGOTIC TWINS

	R-R	R-L	TOTAL
Without L relatives	105	25	130
With L relatives	26	22	48
Total	131	47	178

$\chi^2 = 12.8$

DIZYGOTIC TWINS

	R-R	R-L	TOTAL
Without L relatives	84	12	96
With L relatives	16	15	31
Total	100	27	127

$\chi^2 = 18.1+$

difference in the frequency of left-handedness in non-twin and twin bearing families. These data are apparently not in accord with LAUTERBACH'S explanation of the greater frequency of left-handedness among twins.

SUMMARY

While our criteria for handedness are by no means perfect, and our classification of left-handers is somewhat arbitrary, the data conclusively indicate the following points. Left-handers are more likely to have left-handed children than are right-handers. The inheritance of left-handedness cannot be explained solely on the basis of a single pair of genetic factors. There is considerable evidence that handedness is a graded or quantitative trait. Left-handedness occurs more frequently in both monozygotic and dizygotic twins than in the single born, this being due to the relatively frequent occurrence of pairs of whom one member is right-handed and the other left-handed. In both monozygotic and dizygotic twins, pairs in which one member is left-handed have a significantly higher percentage of left-

handed relatives than do pairs composed only of right-handers. Twin bearing families show no greater incidence of left-handedness than do non-twin bearing families.

The following hypothesis may account for the excess of left-handers among twins. As handedness is a quantitative trait, many individuals are genotypically intermediate, that is, not strongly biased in either direction.

TABLE 7*

Two-by-two tables showing comparative frequencies of left- and right-handedness in twin bearing and non-twin bearing families.

A

	NON-TWIN FAMILIES	FAMILIES OF IDENTICAL TWINS	TOTALS
Right-handed	3279	978	4257
Left-handed	263	56	319
Totals	3542	1034	4576

$\chi^2 = 4.9$

B

	NON-TWIN FAMILIES	FAMILIES OF IDENTICAL TWINS	TOTALS
Right-handed	3279	1128	4407
Left-handed	263	98	361
Totals	3542	1226	4768

$\chi^2 = .42$

C

	NON-TWIN FAMILIES	FAMILIES OF FRATERNAL TWINS	TOTALS
Right-handed	3279	509	3788
Left-handed	263	34	297
Totals	3542	543	4085

$\chi^2 = .90$

D

	NON-TWIN FAMILIES	FAMILIES OF FRATERNAL TWINS	TOTALS
Right-handed	3279	716	3995
Left-handed	263	63	326
Totals	3542	779	4321

$\chi^2 = .40$

* In tables A and C, the twins are not included. In B, R-R pairs are included as right-handed individuals and R-L and L-L pairs as left-handed individuals. In D, R-R pairs are included as two right-handed individuals, L-L pairs as two left-handed individuals, and R-L pairs as having one right-hander and one left-hander.

The handedness of such individuals can easily be shifted one way or the other by environmental conditions. In twins, intra-uterine circumstances, such as position and crowding may condition the handedness of such individuals, resulting in one being left-handed and the other right-handed. Individuals genotypically strongly biased for either right- or left-handedness, would not be shifted by environmental circumstances. Thus identical twins genotypically strongly right-handed or left-handed show no intra-pair differences in handedness. Fraternal twins, having different genotypes, may show intra-pair differences in handedness, both on account of

different genotypes and on account of the environmental conditioning of genotypically intermediate individuals. We should expect more left-handedness among the relatives of genotypically intermediate twins than among the relatives of genotypic right-handers. Our data are in harmony with the above conditions.

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CHANCES OF ESTABLISHING THE NON-IDENTITY OF BIOVULAR TWINS, WITH SPECIAL REFERENCE TO INDIVIDUALITY TESTS OF THE BLOOD

ALEXANDER S. WIENER AND IRVING L. LEFF

Serological Laboratory of the Office of the Chief Medical Examiner of the City of New York

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BECAUSE of recent interest in identical and fraternal twins as a means of establishing the hereditary nature of various characters in human beings, there is need for reliable methods of distinguishing the two sorts of twins. In general, if a pair of twins differs in sex or in any other trait, the hereditary nature of which is established, they cannot possibly be monovular. On the other hand, the fact that two twins resemble each other, belong to the same sex and are alike with regard to a large number of hereditary characters, does not absolutely prove them to be monovular, though this would become highly probable.

Aside from sex, the only characters shared by all human beings and transmitted in a simple fashion according to the Mendelian laws are the agglutinogens in the red blood cells. Two of these, designated as A and B, respectively, and discovered by LANDSTEINER in 1900–1901, are transmitted by means of three allelic genes, A, B and R (BERNSTEIN theory) and give rise to four varieties of blood, O, A, B and AB. The existence of two sorts of A agglutinogen, A_1 and A_2, gives rise to subgroups in groups A and AB and increases the number of classes to six. Two additional agglutinogens, M and N, discovered by LANDSTEINER and LEVINE in 1927, are transmitted by a single pair of allelic genes, M and N, and in combination produce three types of blood, M, N and MN. Since the agglutinogens M and N are independent of A and B, with the aid of the five agglutinogens A_1, A_2, B, M and N, 18 types of human blood can be distinguished. Other agglutinogens have also been described but these are not yet readily available and for a discussion of these, the reader is referred to the review of WIENER (1939).

WIENER (1935) gave the formula for the chance that a pair of biovular twins will differ with respect to the agglutinogens M and N. While the formulas for the other cases were not given, the method of deriving them was indicated. In a recent paper by KOMATU (1939), formulas for the chances of recognizing biovular twins by means of the agglutinogens of human blood are given. All but one of these formulas are incorrect. The correct formula deals with the agglutinogens M and N and agrees with the one previously published by WIENER (1935). It is our purpose to indicate

the errors in the formulas of KOMATU and to give the derivation of the correct formulas.

SINGLE PAIR OF ALLELIC GENES, ONE OF WHICH IS DOMINANT

One of the simplest cases to consider is that of a simple Mendelian dominant, for example, any one of the four agglutinogens A, B, M or N, taken alone.

Let us consider a pair of allelic genes, Q and q, where Q is dominant. These give rise to three genotypes, $Q\,Q$, $Q\,q$, and $q\,q$, the first two corresponding to the type **Q**, and the last one to type **q**. If we take the frequency in the general population of the gene $Q = \mathrm{u}$, and the frequency of the gene $q = \mathrm{v}$, where $\mathrm{u}+\mathrm{v} = 1$ or 100 percent, then it is a simple matter to calculate the frequencies of the three genotypes as follows:

$$Q\,Q = \mathrm{u}^2$$

$$Q\,q = 2\mathrm{uv}$$

$$q\,q = \mathrm{v}^2$$

There are six different matings possible and the frequency of each mating in terms of u and v is readily determined by inspection (table 1). In four of the matings all the children belong to a single phenotype, so that in these matings there is no possibility of recognizing fraternal twins. In the mating, $Q\,q \times Q\,q$, $\frac{3}{4}$ of the children belong to type **Q** and $\frac{1}{4}$ to type **q**.

TABLE 1

Chances of recognizing fraternal twins by a character transmitted as a simple Mendelian dominant.

MATING	FREQUENCY OF MATING	FREQUENCY OF CHILDREN OF TYPE Q	FREQUENCY OF CHILDREN OF TYPE q	CHANCE OF NON-IDENTITY OF TYPES OF FRATERNAL TWINS
1) $Q\,Q \times Q\,Q$	u^4	100	0	0
2) $Q\,Q \times Q\,q$	$4\mathrm{u}^3\mathrm{v}$	100	0	0
3) $Q\,q \times Q\,q$	$4\mathrm{u}^2\mathrm{v}^2$	75	25	$\frac{3}{2}\mathrm{u}^2\mathrm{v}^2$
4) $Q\,Q \times q\,q$	$2\mathrm{u}^2\mathrm{v}^2$	100	0	0
5) $Q\,q \times q\,q$	$4\mathrm{uv}^3$	50	50	$2\mathrm{uv}^3$
6) $q\,q \times q\,q$	v^4	0	100	0
All Combined	100			$\frac{3}{2}\mathrm{u}^2\mathrm{v}^2+2\mathrm{uv}^3$

The chance that both members of a pair of fraternal twins from such a mating belong to type **Q** is $(\frac{3}{4})^2$ the chance that they both belong to type **q** is $(\frac{1}{4})^2$. Therefore, the chance that two fraternal twins from this mating belong to different types is $1 - [(\frac{3}{4})^2+(\frac{1}{4})^2] = \frac{3}{8}$. Since the frequency of the mating is $4\mathrm{u}^2\mathrm{v}^2$, the contribution of this case to the chance of identifying non-identical twins is $\frac{3}{2}\mathrm{u}^2\mathrm{v}^2$. Similarly, in the mating $Q\,q \times q\,q$, the chance

that one member of a pair of biovular twins belongs to type **Q** and the other to type **q** is $2uv^3$. Therefore, if P_Q represents the chance, in general, of recognizing fraternal twins with the aid of a simple Mendelian dominant,

Then, $P_Q = \frac{3}{2}u^2v^2 + 2uv^3$

$= \frac{1}{2}uv^2(3u+4v)$.

Since, $u = 1 - v$

$$P_Q = \frac{1}{2}uv^2(3+v). \qquad (1)$$

For comparison the formula as reported by KOMATU is given:

$$P_Q = \frac{2uv^2(1 + 3v)}{(1 + v)^2}. \qquad (2)$$

KOMATU does not relate how he arrived at this formula, so that it is not possible to point out the mistake in his derivation. However, as will be shown later, his various formulas, including the one given above, contradict one another, and therefore cannot all be correct.

From equation (1) it is evident that the value of P_Q depends upon the frequencies of the genes (or types). If all the individuals in a population are alike with respect to the trait in question, then either u or $v = 0$, and P_Q has its minimum value, namely, zero. It is of interest to determine the maximum value that P_Q can attain. This is done by setting

$$\frac{dP_Q}{dv} = 0 \cdot$$

Since $u = 1 - v$, $P_Q = \frac{1}{2}v^2(3+v)(1-v)$

Therefore, $$\frac{dP_Q}{dv} = \frac{1}{2}[6v - 6v^2 - 4v^3] = 0 \cdot$$

$2v^2 + 3v - 3 = 0$.

Hence, $v = -\frac{3}{4} + \frac{1}{4}\sqrt{33} = 69.6$ percent.

And $u = 30.4$ percent.

The maximum value of $P_Q = 27.2$ percent.

The distribution of the types for this value of P_Q is as follows:

Q = 51.6 percent and **q** = 48.4 percent.

INDEPENDENT FACTORS

If the chances of recognizing fraternal twins by each of two or more independent hereditary properties is known, then it is a simple matter to calculate the chance of solving this type of problem by the combined use of all the characters. Thus, let $P_1, P_2, P_3 \cdots P_n$ represent the individual chances for n different traits, respectively. The chance that the fraternal

twins resemble each other with regard to every one of these characters is

$$(1-P_1)(1-P_2)(1-P_3)\cdot\cdot\cdot(1-P_n).$$

Therefore the chance that they differ with regard to at least one character, which is the probability P that we want to derive, is as follows:

$$P=1-(1-P_1)(1-P_2)(1-P_3)\cdot\cdot\cdot(1-P_n). \tag{3}$$

The maximum value of P for n independent Mendelian dominant characteristics is $1-(1-0.272)^n$.

SINGLE PAIR OF ALLELIC GENES, WITHOUT DOMINANCE

As has already been shown, the agglutinogens M and N are transmitted by a pair of alleles, *M* and *N*. Three genotypes are possible, *MM*, *NN* and *MN*, corresponding to types **M**, **N** and **MN**, respectively.

It is a simple matter to derive the value of P_{MN} by using the same method as that given for a simple Mendelian dominant. From table 2, it is evident that

$$P_{MN}=2m^3n+2mn^3+\tfrac{5}{2}m^2n^2. \tag{4}$$

where m and n represent the frequencies of genes *M* and *N*, respectively. Since $m+n=1$, equation (4) reduces to

TABLE 2

Chances of recognizing fraternal twins by the agglutinogens M and N.

MATING	FREQUENCY OF MATING	FREQUENCY OF CHILDREN OF TYPE			CHANCE OF NON-IDENTITY OF TYPES OF FRATERNAL TWINS
		M	N	MN	
1) *MM×MM*	m^4	100	0	0	0
2) *MM×NN*	$2m^2n^2$	0	0	100	0
3) *MM×MN*	$4m^3n$	50	0	50	$2m^3n$
4) *NN×NN*	n^4	0	100	0	0
5) *NN×MN*	$4mn^3$	0	50	50	$2mn^3$
6) *MN×MN*	$4m^2n^2$	25	25	50	$\frac{5}{2}m^2n^2$

$$\begin{aligned} P_{MN} &= 2mn(m^2+n^2)+{}^{5}m^2n^2 \\ &= 2mn(1-2mn)+\tfrac{5}{2}m^2n^2 \\ &= 2mn-\tfrac{3}{2}m^2n^2 \qquad (5) \\ P_{MN} &= \tfrac{1}{2}mn(4-3mn). \end{aligned}$$

In this case, as in that of a simple Mendelian dominant, the value of P_{MN} becomes zero if the frequency of either gene is zero. Its maximum value is obtained by setting $\frac{dP_{MN}}{dm}=0$. Since $n=1-m$,

$$P_{MN} = \tfrac{1}{2}m(1 - m)[4 - 3m(1 - m)]$$

$$\frac{dP_{MN}}{dm} = 2 - 7m + 9m^2 - 6m^3 = 0.$$

Therefore, $(1 - 2m)(2 - 3m + 3m^2) = 0$.

So that $m = \frac{1}{2}$ and $n = \frac{1}{2}$.

Hence P_{MN} has its maximum value when the distribution of the types in the population is **M** = 25 percent, **N** = 25 percent and **MN** = 50 percent, and the maximum value of P_{MN} is $\frac{13}{32}$ or 40.6 percent.

In most white populations thus far examined the frequency (m) of gene *M* is approximately 55 percent; in such populations the value of P_{MN} = 40.3 percent, which is very close to the maximum value.

MULTIPLE ALLELIC GENES

As has been indicated above, the four blood groups furnish an example of heredity by means of multiple allelic genes in man. If p, q and r represent the frequencies of the genes *A*, *B* and *R*, respectively, then, the value of P_{AB} is derived as shown in table 3.

TABLE 3

Chances of recognizing fraternal twins by the four blood groups.

MATING	FREQUENCY OF MATING	FREQUENCY OF CHILDREN OF GROUP				CHANCE OF NON-IDENTITY OF GROUPS OF FRATERNAL TWINS
		O	A	B	AB	
1) *RR×RR*	r^4	100	0	0	0	0
2) *RR×AA*	$2p^2r^2$	0	100	0	0	0
3) *RR×AR*	$4pr^3$	50	50	0	0	$2pr^3$
4) *RR×BB*	$2q^2r^2$	0	0	100	0	0
5) *RR×BR*	$4qr^3$	50	0	50	0	$2qr^3$
6) *RR×AB*	$4pqr^2$	0	50	50	0	$2pqr^2$
7) *AA×AA*	p^4	0	100	0	0	0
8) *AA×AR*	$4p^3r$	0	100	0	0	0
9) *AA×BB*	$2p^2q^2$	0	0	0	100	0
10) *AA×BR*	$4p^2qr$	0	50	0	50	$2p^2qr$
11) *AA×AB*	$4p^3q$	0	50	0	50	$2p^3q$
12) *AR×AR*	$4p^2r^2$	25	75	0	0	$\frac{3}{2}p^2r^2$
13) *AR×BB*	$4pq^2r$	0	0	50	50	$2pq^2r$
14) *AR×BR*	$8pqr^2$	25	25	25	25	$6pqr^2$
15) *AR×AB*	$8p^2qr$	0	50	25	25	$5p^2qr$
16) *BB×BB*	q^4	0	0	100	0	0
17) *BB×BR*	$4q^3r$	0	0	100	0	0
18) *BB×AB*	$4pq^3$	0	0	50	50	$2pq^3$
19) *BR×BR*	$4q^2r^2$	25	0	75	0	$\frac{3}{2}q^2r^2$
20) *BR×AB*	$8pq^2r$	0	25	50	25	$5pq^2r$
21) *AB×AB*	$4p^2q^2$	0	25	25	50	$\frac{5}{2}p^2q^2$

By combining terms, we have

$$P_{AB} = 2pr^3 + 2qr^3 + \tfrac{3}{2}p^2r^2 + \tfrac{3}{2}q^2r^2 + 8pqr^2 + 7p^2qr + 7pq^2r + 2p^3q + 2pq^3 + \tfrac{5}{2}p^2q^2 = r^2(2pr + 2qr + \tfrac{3}{2}p^2 + \tfrac{3}{2}q^2 + 8pq) + pq(7pr + 7qr + 2p^2 + 2q^2 + \tfrac{5}{2}pq). \quad (6)$$

But $p + q + r = 1$.

And $p^2 + q^2 + r^2 + 2pq + 2pr + 2qr = 1$.

Therefore, $P_{AB} = r^2(1 + \tfrac{1}{2}p^2 + \tfrac{1}{2}q^2 - r^2 + 6pq) + pq(2 - 2r^2 + 3pr + 3qr - \tfrac{3}{2}pq)$.

$$P_{AB} = r^2(1 - r^2) + \tfrac{1}{2}r^2(p^2 + 12pq + q^2) + 2pq(1 - r^2) + \tfrac{1}{2}pq(6pr + 6qr - 3pq).$$

$$P_{AB} = r^2(1 - r^2) + \tfrac{1}{2}r^2[(p + q)^2 + 10pq] + 2pq(1 - r^2) + \tfrac{1}{2}pq[6r(p + q + r) - 6r^2 - 3pq].$$

$$P_{AB} = r^2(1 - r)(1 + r) + \tfrac{1}{2}r^2(1 - r)^2 + 5pqr^2 + 2pq - 2pqr^2 + \tfrac{1}{2}pq(6r - 6r^2 - 3pq).$$

$$P_{AB} = r^2(1 - r)[(1 + r) + \tfrac{1}{2}(1 - r)] + 3pqr^2 + 2pq + 3pqr - 3pqr^2 - \tfrac{3}{2}p^2q^2.$$

Therefore, $P_{AB} = \tfrac{1}{2}r^2(1 - r)(3 + r) + \tfrac{1}{2}pq(4 - 3pq + 6r)$. (7)

For comparison, the following formula, given by KOMATU, is offered:

$$P_{AB} = 2r^2(1 - r)(3 - r) + 2pq(2 + r - 4r^2) - \tfrac{3}{2}p^2q^2 + 14r^4\left[\left(\frac{p}{p + 2r}\right)^2 + \left(\frac{q}{q + 2r}\right)^2\right] - \frac{8pqr^4}{(p + 2r)(q + 2r)} - \frac{2pr^2}{p + 2r}[2r(4p + r) + q(p + 2q)] - \frac{2qr^2}{q + 2r}[2r(4q + r) + p(q + 2p)]. \quad (8)$$

The first thing that strikes one about KOMATU's formula is that his final formula is far more complicated than the unsimplified sum given in equation (6) from which the formula (7) was derived. That the formula (8) is incorrect can be shown by setting $r = 0$, in which case the formula should reduce to equation (5) for the agglutinogens M and N, substituting the letters, m and n, for p and q, respectively. Whereas, according to equation (5), P_{AB} should equal $\tfrac{1}{2}pq(4-3pq)$ when $r = 0$, on the other hand, from formula (8), P_{AB} reduces to $\tfrac{1}{2}pq(8-3pq)$. Since KOMATU himself asserts that equation (5) is correct, on this basis, formula (8) must be

invalid. On the other hand, formula (7) satisfies the requirement that it reduce the formula (5) when $r = 0$. In a similar manner, the equation for P_{AB} should reduce to the formula for P_Q when either p or $q = 0$, the letter r in the formula obtained in this manner corresponding to the letter v in the formula for P_Q, and $1 - r$ corresponding to u. By inspection it will be found that when either p or $q = 0$, formula (7) reduces to formula (1). On the other hand, KOMATU's formula (8) for P_{AB} does not reduce to his own formula for P_Q.

It is of interest to determine what contribution to the chance of identifying fraternal twins is made by the subgroups of A and AB. According to the theory of THOMSEN, FRIEDENREICH and WORSAAE (1930) the hereditary transmission of the groups, including the subgroups, is by means of four allelic genes, A_1, A_2, B and R where A_1, A_2 and B are dominant over R, and A_1 is dominant over A_2.

In table 4, we have summarized the contribution to the chance of recognizing fraternal twins of each mating listed there. Not every possible mating is tabulated; those which contribute nothing to the chances have been omitted in order to shorten the table. The value of $P_{A_1A_2B}$ is obtained by adding all the terms in the last column of the table. To simplify the resulting expression, the best procedure is to ascertain by how much $P_{A_1A_2B}$ exceeds P_{AB}, bearing in mind that $p_1 + p_2 = p$. The following expression is obtained:

$$\begin{aligned} P_{A_1A_2B} = P_{AB} &+ 4p_1p_2r^2 + 3p_1p_2qr + p_1^2p_2q + 2p_1p_2^2q \\ &+ 3p_1p_2q^2 + 6p_1p_2^2r + \tfrac{3}{2}p_1^2p_2^2 + 3p_1^2p_2r + 2p_1p_2^3. \end{aligned} \tag{9}$$

Simplifying, we obtain the following formula:

$$P_{A_1A_2B} = P_{AB} + p_1p_2(2p_2 + 3r + r^2 + p_1q + 3q^2 + p_2r - \tfrac{1}{2}p_1p_2). \tag{10}$$

Obviously, this formula reduces to P_{AB} when either p_1 or $p_2 = 0$, as is to be expected.

It is of interest to apply formulas (7) and (10) to an actual case. As an example we can determine what the values of P_{AB} and $P_{A_1A_2B}$ are for the population studied by WIENER and ROTHBERG (1933). These investigators found the distribution of the groups and subgroups in a series of approximately 900 individuals in New York City to be:

$$O = 44.6\%,\ A_1 = 25.6\%,\ A_2 = 12.0\%,\ B = 13.6\%,\ A_1B = 3.1\%$$
$$\text{and } A_2B = 1.2\%.$$

Therefore, by applying the formulas for the frequency of the genes given by WIENER (1939) we have:

$$p_1 = 15.3\%,\ p_2 = 8.4\%,\ q = 9.3\% \text{ and } r = 66.8\%.$$

Substituting the values $p = 0.237$, $q = .093$ and $r = 0.668$ in formula (7),

TABLE 4

Chances of recognizing fraternal twins by the six groups, O, A_1, A_2, B, A_1B *and* A_2B.

MATING	FREQUENCY OF MATING	FREQUENCY OF CHILDREN OF GROUP						CHANCE OF NON-IDENTITY OF GROUPS OF FRATERNAL TWINS
		O	A_1	A_2	B	A_1B	A_2B	
1) $RR \times A_1A_2$	$4p_1p_2r^2$	0	50	50	0	0	0	$2p_1p_2r^2$
2) $RR \times A_1R$	$4p_1r^3$	50	50	0	0	0	0	$2p_1r^3$
3) $RR \times A_2R$	$4p_2r^3$	50	0	50	0	0	0	$2p_2r^3$
4) $RR \times BR$	$4qr^3$	50	0	0	50	0	0	$2qr^3$
5) $RR \times A_1B$	$4p_1qr^2$	0	50	0	50	0	0	$2p_1qr^2$
6) $RR \times A_2B$	$4p_2qr^2$	0	0	50	50	0	0	$2p_2qr^2$
7) $A_1A_1 \times BR$	$4p_1^2qr$	0	50	0	0	50	0	$2p_1^2qr$
8) $A_1A_1 \times A_1B$	$4p_1^3q$	0	50	0	0	50	0	$2p_1^3q$
9) $A_1A_1 \times A_2B$	$4p_1^2p_2q$	0	50	0	0	50	0	$2p_1^2p_2q$
10) $A_1A_2 \times A_1A_2$	$4p_1^2p_2^2$	0	75	25	0	0	0	$\frac{3}{2}p_1^2p_2^2$
11) $A_1A_2 \times A_1R$	$8p_1^2p_2r$	0	75	25	0	0	0	$3p_1^2p_2r$
12) $A_1A_2 \times A_2A_2$	$4p_1p_2^3$	0	50	50	0	0	0	$2p_1p_2^3$
13) $A_1A_2 \times A_2R$	$8p_1p_2^2r$	0	50	50	0	0	0	$4p_1p_2^2r$
14) $A_1A_2 \times BB$	$4p_1p_2q^2$	0	0	0	0	50	50	$2p_1p_2q^2$
15) $A_1A_2 \times BR$	$8p_1p_2qr$	0	25	25	0	25	25	$6p_1p_2qr$
16) $A_1A_2 \times A_1B$	$8p_1^2p_2q$	0	50	0	0	25	25	$5p_1^2p_2q$
17) $A_1A_2 \times A_2B$	$8p_1p_2^2q$	0	25	25	0	25	25	$6p_1p_2^2q$
18) $A_1R \times A_1R$	$4p_1^2r^2$	25	75	0	0	0	0	$\frac{3}{2}p_1^2r^2$
19) $A_1R \times A_2A_2$	$4p_1p_2^2r$	0	50	50	0	0	0	$2p_1p_2^2r$
20) $A_1R \times A_2R$	$8p_1p_2r^2$	25	50	25	0	0	0	$5p_1p_2r^2$
21) $A_1R \times BB$	$4p_1q^2r$	0	0	0	50	50	0	$2p_1q^2r$
22) $A_1R \times BR$	$8p_1qr^2$	25	25	0	25	25	0	$6p_1qr^2$
23) $A_1R \times A_1B$	$8p_1^2qr$	0	50	0	25	25	0	$5p_1^2qr$
24) $A_1R \times A_2B$	$8p_1p_2qr$	0	25	25	25	25	0	$6p_1p_2qr$
25) $A_2A_2 \times BR$	$4p_2^2qr$	0	0	50	0	0	50	$2p_2^2qr$
26) $A_2A_2 \times A_1B$	$4p_1p_2^2q$	0	50	0	0	0	50	$2p_1p_2^2q$
27) $A_2A_2 \times A_2B$	$4p_2^3q$	0	0	50	0	0	50	$2p_2^3q$
28) $A_2R \times A_2R$	$4p_2^2r_2$	25	0	75	0	0	0	$\frac{3}{2}p_2^2r^2$
29) $A_2R \times BB$	$4p_2q^2r$	0	0	0	50	0	50	$2p_2q^2r$
30) $A_2R \times BR$	$8p_2qr^2$	25	0	25	25	0	25	$6p_2qr^2$
31) $A_2R \times A_1B$	$8p_1p_2qr$	0	50	0	25	0	25	$5p_1p_2qr$
32) $A_2R \times A_2B$	$8p_2^2qr$	0	0	50	25	0	25	$5p_2^2qr$
33) $BB \times A_1B$	$4p_1q^3$	0	0	0	50	50	0	$2p_1q^3$
34) $BB \times A_2B$	$4p_2q^3$	0	0	0	50	0	50	$2p_2q^3$
35) $BR \times BR$	$4q^2r^2$	25	0	0	75	0	0	$\frac{3}{2}q^2r^2$
36) $BR \times A_1B$	$8p_1q^2r$	0	25	0	50	25	0	$5p_1q^2r$
37) $BR \times A_2B$	$8p_2q^2r$	0	0	25	50	0	25	$5p_2q^2r$
38) $A_1B \times A_1B$	$4p_1^2q^2$	0	25	0	25	50	0	$\frac{5}{2}p_1^2q^2$
39) $A_1B \times A_2B$	$8p_1p_2q^2$	0	25	0	25	25	25	$6p_1p_2q^2$
40) $A_2B \times A_2B$	$4p_2^2q^2$	0	0	25	25	0	50	$\frac{5}{2}p_2^2q^2$

we find that $P_{AB} = 35.9$ percent. From equation (10) and the fact that $p_1 = 15.3$ percent and $p_2 = 8.4$ percent, we find that $P_{A_1A_2B} = 0.359 + 0.035 = 39.4$ percent.

It is a simple matter now to calculate how frequently fraternal twins can be diagnosed if one takes into account the sex of the twins, the blood groups, subgroups and MN-types. From equation (3), we have:

$P = 1-(1-0.5)(1-0.394)(1-0.403) = 0.819$, taking the chance of diagnosing fraternal twins by means of sex equal to 0.5, $P_{A_1A_2B} = 0.394$ and $P_{MN} = 0.403$.

SUMMARY

Formulas have been derived for the chances of proving that a pair of fraternal twins are biovular (1) by means of characters transmitted as simple Mendelian dominants, (2) by the four blood groups, O, A, B and AB, (3) by the agglutinogens, M and N, and (4) by the subgroups of groups A and AB. The formulas reported by KOMATU were found to be different from those derived by the present writers. It was demonstrated that KOMATU's formulas must be incorrect since they contradict one another.

Using the formulas derived by the present writers, it was calculated that for a population such as the one in New York City, 81.9 percent of fraternal twins could be diagnosed as biovular by taking into account their sex, blood groups, subgroups and MN types.

ADDENDUM

As further evidence of the accuracy of our formulas (as well as the inaccuracy of KOMATU's formulas), we cite a paper by RIFE (1939) which was brought to our attention while the present communication was in press. RIFE has also calculated the frequency of discordance in fraternal twins in various simple types of heredity, though he does not give the general formulas. He finds in the case of a single pair of alleles, one of which is dominant, the maximum value of P_Q to be about 27.2 percent, which coincides precisely with our own results. His value for P_{MN} (about 40 percent) is the same as ours (40.3) and his value for P_{AB} (35 percent) also closely approximates our own (35.9).

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A COLORIMETRIC STUDY OF GENIC EFFECT ON GUINEA-PIG COAT COLOR

GERTRUDE HEIDENTHAL
The University of Chicago, Chicago, Illinois

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INTRODUCTION

ONE approach to the study of gene physiology is obviously a description of the end-products of genic interaction. Estimates of quantity and an elucidation of any qualitative differences between or within allelic series should aid in the establishment of a general theory and thus in an understanding of the links between gene and phenotype. With respect to pigmentation in the guinea-pig, it has seemed desirable to continue the attack started by RUSSELL (1939) on the quantitative aspects of the problem although the need for qualitative chemical analysis cannot be denied since, at the present time, we have only rather meager suggestions as to differences in kind. We know, for example, that in general the yellow pigments from mammalian hair are more readily soluble in cold dilute alkalis than are the sepia melanins, although information on other differences between these pigments, and possible variations within the sepia and yellow end-products are still lacking. WRIGHT'S work (1927) with the Milton-Bradley color-wheel led him to the suggestion that the differences between red and yellow grades were more than simple quantitative variations since the proportion of orange required to match red skins (using black, white, yellow and orange) was greater than that needed for the yellow ones. The more recent work of DANIEL (1938) giving spectrophotometric measurements of solutions of sepia pigment from the hair of the mouse has indicated, however, that qualitative differences probably do not exist within the genotypes tested (combinations of the A, B, C series). That care must be exercised in drawing conclusions from these data is indicated by the author's statement to the effect that there is still some uncertainty concerning the method.

Although the quantitative measurement of hair pigments is fraught with difficulties, some rather satisfactory attempts have recently been made by EINSELE (1937) and DUNN and EINSELE (1938) for the mouse, and by RUSSELL (1939) for the guinea-pig. In the latter case, advantage was taken of an observation made by DURHAM (1904) and others that yellow pigment is rather readily soluble in cold, dilute alkali. RUSSELL, accordingly, was able to develop a colorimetric method for the determination of intensity differences among animals with yellow fur. Since the sepia pigments are relatively resistant to this treatment the same method could

not be used for this color quality. She therefore developed another technique by which the pigment was first separated from a known weight of hair, essentially according to the method of EINSELE, and then titrated with $KMnO_4$ to determine the amount of this substance which could be reduced by the isolated pigment. EINSELE, meanwhile, had found that the dark pigments of mouse hair could be made to dissolve by boiling in solutions of KOH (1937). The present investigation was undertaken for the purpose of extending the observations on the yellow series and of developing a colorimetric method for the sepia genotypes. It was also hoped that if the sepia pigments could be made to go into solution, the resulting color quality might be sufficiently similar to that of yellow to warrant quantitative comparisons between the two series. A colorimetric method for the sepia series has been developed, but the solutions were found to have a somewhat duskier hue than those of the yellow series. Thus comparisons between the two qualities are not available.

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MATERIALS AND METHODS

The major color-factors of the guinea-pig have been so thoroughly investigated by WRIGHT (1915, 1916, 1917, 1923, 1925, 1927) that it is hardly necessary to describe the stocks at length. Of the seven major series of alleles, readings were made on compounds of the members of four. These include E, e in which E governs the production of sepia, and e of yellow quality; the albino series C, c^k, c^d, c^r, c^a the members of which modify the intensity of both yellow and sepia; the P, p series in which the homozygous recessive dilutes sepia but has no effect on yellow; the F, f series in which ff reduces yellow but has no effect on sepia except in combinations involving $E-ffpp$ in which case the animals are of a low grade of yellow or white. Practical considerations have made it impossible up to the present time to introduce the various combinations into a single isogenic stock although the desirability of this is evident.

The routine procedure in the color experiments was to grade each animal within a day or so after birth by means of two qualitatively different sets of pelts standardized according to graduated steps of intensity of yellow and sepia, respectively. Genotypes were assigned on the basis of color quality and intensity, the genotypes of the parents, and in some cases, breeding tests. Three to four weeks after birth, hair was clipped to approximately equal length of base from the mid-dorsal region of the body and stored in manila envelopes away from any intense source of light. The time between collection and use varied from a few weeks to a year.

The yellow series

The technique for the yellow genotypes was substantially that followed by RUSSELL. The hair was first washed in two changes of cold ether and dried. Samples weighing 100 mgs (weighed to the nearest mg) were then placed in 10 cc of a .5N solution of NaOH and allowed to stand for five days. The unhydrolyzed hair was removed by filtration and the resulting colored solutions were compared to each other in a colorimeter (Klett biometer). As explained by RUSSELL, the intensity of color seen in the field of a colorimeter depends upon the intensity of the colored solution and the height of the column through which the light passes. Furthermore, if two intensities are matched, intensity 1:intensity 2 = height 2: height 1. It follows from this relationship that if height 1 is fixed arbitrarily (for example, at 15 mm) and height 2 is determined by matching the intensity of solution 2 against that of solution 1, the ratio of these two heights will give a measure of the relative intensities of the two solutions.

Before a given series of comparisons was made, the colorimeter was calibrated by placing a portion of a given standard in each of the two colorimeter cups. These identical samples were read against each other in the manner described above. The colorimeter light was adjusted until it was possible to secure five consecutive readings in which height 2 fell within a range of $15 \pm .4$ mm (height $1 = 15$ mm).

The determinations for a given animal were made as follows. The duplicate experimental solutions from a particular sample were first compared to each other by means of ten consecutive, independent readings. Then each was compared with one of a series of standards made by diluting an intense solution from the hair of a single yellow adult guinea-pig (grade 9 and constitution $eeC-F-$) with the .5N solution of NaOH in the proportions of 1:0, 2:1, 1:1, 1:2, 1:6. Thus for a given animal there were two comparisons with standard, each involving ten readings, hence ten ratios (h1/h2). The arithmetic average of the means of these two series of ratios was taken as the colorimetric value (CV) for the animal.

The only departure from the above procedure was in the case of grades 0 and 1, at the lower end of the intensity scale. Solutions from these grades were so light that the two sides of the colorimeter field were indistinguishable within a mean range of about 4 mm. The preparations were, however, not colorless but of a distinctly yellow tinge and it was therefore desirable to have at least an approximate value for them on the colorimetric scale. The practice was, accordingly, to use the lightest standard and with that to make four estimates each of the upper and lower limits of the indistinguishable band. The mid-point of this range was taken as the final determination.

The sepia series

The method for separating the sepia pigment from the keratin structure was essentially that developed by EINSELE (1937) and later used by RUSSELL. The keratin was hydrolyzed in 6N HCl and the resulting suspensions containing the pigment granules, were centrifuged and washed in water to separate the hydrolysate from the pigment (plus the small quantities of occluded impurities). For every animal in the present work two samples, each weighing 100 mgs, were treated in this manner. Considerable difficulty in separation was experienced with grades 10–12 but by prolonged and repeated centrifugation and careful slowing of the centrifuge, success was finally attained in four cases. These experiences agree in general with those of RUSSELL who circumvented the difficulty with centrifugation by adding weighed quantities of the coarsely granular pigment from black animals, to the suspensions, and was thus able to clear the hydrolysate, since the lighter pigments were precipitated by occlusion. The author did not use this method for the following reason. The data secured by RUSSELL on the percentage weight of melanin in sepia hair indicated that the proportion of pigment in these genotypes is very small (a mean of 1.37 percent for the genotype $E-c^dc^aP-$). Thus for the lighter grades reliable weights could not be attained. The experimental error in adding extra black pigment for purposes of centrifugation might, therefore, tend to obscure the true values for the pale genotypes. This may account to some extent for the fluctuating permanganate values at the lower end of the scale.

A different procedure from that used by RUSSELL was followed after the technique of separation had been completed. The pigment (two samples from each animal) was put into solution by boiling for $1\frac{3}{4}$ to $2\frac{1}{2}$ hours in a reflux condenser with a .2N solution of KOH. The quantity of alkali was varied from 25 cc to 100 cc in accordance with the amount of pigment judged to be present on the basis of grade. This procedure was followed in order that all solutions might be of an intensity suitable for reading against a standard made by dissolving the pigment in 100 mgs of hair from an animal of grade 19 and constitution $E-c^rc^rP-$, in 100 cc of the solution of KOH. The alkali was added directly to the pigment plus the small amount of water left after the last decantation. Consequent slight variation in pH could have had little if any effect on the readings since it will be evident from a later analysis that the errors between two samples from the same animal were small.

Preliminary work indicated that it was not always easy to tell exactly at what point the pigment went into solution. At times the "solution" would appear to be clear, yet filtration and centrifugation indicated that small particles were still in suspension. The practice, therefore, was to boil the suspensions for about one-half hour beyond the time when, to the naked

eye, they looked clear. In no case did subsequent filtration or centrifugation indicate any detectable, undissolved granules. Tests of additional boiling were made on 14 of these solutions. In each case, two samples of sufficient quantity to fill the colorimeter cups were read against each other. One of these was then saved and all of the remaining solution was boiled for an additional hour. Determinations for the portion which had undergone extra boiling were made against the sample which had been saved. An analysis by Student's method for paired comparisons yielded a value of 1.24 for t. This shows that no significant change in intensity had occurred. It is assumed, therefore, that the technique of boiling beyond the time when the suspensions appeared to have gone into solution had no appreciable effect, at least within the limits indicated.

After the solutions had been adjusted for volume, they were read against the standard ($E-c^rc^rP-$, grade 19) and against each other. As in the yellows, each determination was taken as the mean of ten ratios secured from ten independent readings. The colorimeter value (CV) for a given animal is the arithmetic average of the two means secured from reading the experimental solutions against standard, except for the lower grades in which less than 100 cc of KOH was used. In these cases the average was multiplied by the appropriate factor, to give the true colorimetric value.

The possibility that changes in standard might have occurred was checked at the end of four months (three weeks after the last experimental determinations were made) by preparing two solutions from the same sample of hair which had been used in the preparation of the standard itself. These two solutions when compared with the original standard gave mean readings of 1.076 and 1.063. Such figures indicate that the standard probably had faded slightly in spite of the ordinary precaution of keeping it tightly stoppered in a photographic dark room, except when readings were being made.

A second test for the possible effects of change in standard on the readings was made by examining the determinations within grades 21, 20, 19, 18 (groups having the largest numbers) for trend. The data for each group were trichotomized according to the time when the experiments were performed, and a grand unweighted mean for each of the three periods was determined. These values were 1.137, 1.157, 1.189 for the first, second and third periods, respectively. The numbers are too small for an elaborate statistical study of trend, but the determinations for the three periods are at least consistent with the view that the standard did undergo a slight reduction in intensity. Such a change is not regarded as serious since it must have been small and it will be evident that the variability within both grade and genotype is relatively large.

RESULTS AND DISCUSSION OF RESULTS

Errors of the methods

Some measure of the reliability of the methods is necessary. The errors can be grouped into two classes: (1) the experimental error involved in sampling from the hair, weighing the hair and preparing the solutions for colorimetry, (2) the colorimetric error. The latter was determined directly, in the case of the readings of sample two against sample one, by finding the average variance within the sets of ten readings $(\sum_1^n \sum_1^{10}(v-m)^2/9n)$ where n is the number of comparisons, the v's are the individual readings and the m's are the means of the sets of ten readings. In the case of yellow (101 cases, including all of grades 2–12 and two of grade one) this variance was .00090, indicating a standard deviation of .030. The standard error of the mean of ten readings was thus only .0095 $(=.030/\sqrt{10})$ or 0.95 percent, since the mean of the 101 means was 1.00.

The variance of set means (sample two read directly against sample one) was .00296 $(=\Sigma_1^{101}[m-1.00]^2/_{100})$. This is compounded of the experimental errors in the preparation of both samples $(2\sigma_e^2)$ and the colorimetric error (σ_c^2) which we have found to be .000090 $(=.0095^2)$. The colorimetric error is thus only about three percent of the total error in this case.

In comparisons of sample one with standard, experimental error is involved only once. Thus the colorimetric error should constitute nearly twice as great a proportion of the total as above, viz., $\sigma_c^2/\sigma_e^2+\sigma_c^2$, or 5.9 percent, provided the colorimetric error is the same (on the appropriate scale) as in the comparison with the other sample.

A second test can be obtained from the correlation of the mean direct readings of one sample against the other with the corresponding ratio of the mean readings of each against the standard. If there were no colorimetric error, the correlation (r) would necessarily be perfect. The actual value of r in the yellow series was $.89 \pm .02$. The portion of the total error due to colorimetry can be found as follows. A direct reading of one sample against the other, deviates in a particular case because of the errors in the preparation of both solutions and also because of *one* error of colorimetry. The ratio of the mean readings against standard deviates from one because of the same errors in preparation plus *two* errors of colorimetry. Let $\sigma_{c_1}^2$ equal the squared standard error of colorimetry in this case. It may differ from σ_c^2 since the samples may vary considerably from standard in concentration. The degree of determination by errors of preparation is $\dfrac{2\sigma_e^2}{2\sigma_e^2+\sigma_c^2}=.97$ in the former case and $\dfrac{2\sigma_e^2}{2\sigma_e^2+2\sigma_{c_1}^2}$ in the latter. The correlation coefficient may be equated to the product of the square roots of

these expressions which are the path coefficients measuring the contributions of the common factors. This yields $2\sigma_e^2/2\sigma_e^2+2\sigma_{e_1}^2=.82$. The portion of the variance due to experimental error in a comparison of one sample with standard should agree $(\sigma_e^2/\sigma_e^2+\sigma_{e_1}^2)$ giving 18 percent as the portion of the variance in this case due to colorimetry. This is about three times as large as the estimate derived from the readings of the samples against each other and seems to indicate that errors of colorimetry are much greater in comparisons with standard. Nevertheless the colorimetric error is still a minor portion of the total error. The experimental error in preparing one sample may be estimated as .00144 $(=\frac{1}{2}\ .00296-.00009)$. Since $\sigma_e^2/\sigma_e^2+\sigma_{e_1}^2=.82$, the total error in grading one sample with the standard $(\sigma_e^2+\sigma_{e_1}^2)$ may be estimated as $.00176\left(=\frac{.00144}{.82}\right)\cdot$ The standard deviation of total errors for one sample is thus about 4.2 percent $(=\sqrt{.00176})$. The standard deviation for the average of two samples is thus about 3.0 percent $\left(=\frac{4.2}{\sqrt{2}}\right)\cdot$

A comparable statistical analysis for the sepia genotypes (90 experiments run in duplicate and including grades 10–21) yielded a total variance of .00129 for the means of sets of direct readings of one sample against the other. The variance within the sets of ten readings was .00065 and thus the variance of means, due to colorimetry, was .000065 or about 5.0 percent of the total. This would imply about 9.5 percent determination of the total squared error by colorimetric errors in the case of the reading of one sample against standard, if the colorimetric errors remain the same. As before, however, analysis of the correlation between the direct readings, sample two against sample one, and the ratio of the readings against standard, indicates a larger average colorimetric error in the readings against standard. The correlation coefficient was $.86\pm.03$ in this case. This gives 78 percent $\left(=\frac{.86^2}{.95}\right)$ as the estimated portion of the variance due to experimental errors, in comparisons with standard, and 22 percent (instead of 9.5 percent) as the portion due to colorimetric errors. The total error in a comparison of one sample with standard may be estimated as $.00079\left(=\frac{.000618}{.78}\right)\cdot$ The standard deviation of total errors for one sample is thus about 2.8 percent $(=\sqrt{.00079})$ and the standard deviation for the average of two samples is about 2.0 percent. It should be pointed out that

standard deviations of such a size are a little low for the method as a whole. This is accounted for by the following considerations. All experiments in which the two samples from the same animal were grossly different were repeated. In eight of the nine cases in which this procedure was followed the solutions of the second gave readings within a ten percent range of each other and were, therefore, regarded as reflecting a more accurate value for the animal under experimentation. The most probable source of gross error in such cases was loss of pigment during decantation of the hydrolysate or wash water.

The yellow series

The colorimetric values for the yellow series are given by grade in table 1 and by genotype in table 2 and figure 1. The standard errors in tables 1 and

TABLE 1

A comparison of the colorimetric values for grades of yellow with those estimated by Russell.

GRADE	PRESENT DATA					RUSSELL'S DATA		
	NO. OF ANIMALS	MEAN CV	σ	GENERALIZED σ	S.E.	NO. OF ANIMALS	MEAN CV	GENERALIZED S.E.
12	3	1.849	.156	.348	±.201	1	2.654	±.667
11	6	1.516	.258	.285	.117	9	3.020	.253
10	13	1.460	.276	.275	.076	15	2.872	.186
9	6	1.164	.167	.219	.090	2	2.237	.397
8	5	0.848	.104	.160	.071	6	1.403	.144
7	27	0.618	.101	.116	.022	34	1.039	.045
6	10	0.544	.112	.102	.032	12	0.778	.056
5	7	0.413	.104	.078	.029	17	0.627	.038
4	13	0.235	.054	.044	.012	22	0.552	.030
3	7	0.173	.054	.035	.013	12	0.357	.026
2	2	0.133	.019	.025	.018	4	0.239	.030
1	3	0.064	.015	.020	.012	1	0.122	.031
0	4	0.057	.005	.017	±.008	3	0.120	±.017

2 were calculated in the usual manner but from a generalized standard deviation secured by multiplying the mean for each grade or genotype, as the case might be, by the weighted mean coefficient of variability for all grades or genotypes (weight = n − 1). This procedure was followed in order to find a reasonably reliable measure of variability for groups in which the numbers were so small that standard errors calculated from the actual standard deviations would have little meaning. In grades 10, 7 and 4, classes which occur most frequently, and which, therefore, may be assumed to show the most reliable means, the generalized standard deviation varies only slightly from that calculated directly. The values are reduced in

grades 3–6 but in all other instances they are about the same or larger than the conventional standard deviations.

The uncertainty of the means for very high grades (12, 11, 10) is probably partly due to the fact that animals of high intensity do not hold their original color as do those of lower grades (exclusive of *ff* genotypes). After the data on grades 12 and 11 had been secured, the small samples of hair

TABLE 2

A comparison of the colorimetric values of yellow genotypes with those estimated by Russell.

	PRESENT DATA						RUSSELL'S DATA			
	eeF–						*eeF–*			
C SERIES	NO. OF ANIMALS	MEAN GRADE	MEAN CV	σ	GENERALIZED σ	S.E.	NO. OF ANIMALS	MEAN CV	S.E.	RUSSELL'S TRANSFORMED CV
Cc^k	2	11.5	1.643	.545	.360	±.255	..	.....	±....	.....
Cc^d	9	10.0	1.422	.273	.312	.104	..	.....	....	.....
Cc^r	3	9.3	1.225	.208	.269	.155	..	.....	....	.....
Cc^a	6	10.3	1.530	.301	.335	.137	..	.....	....	.....
C–*	28	10.2	1.450	.300	.318	.060	27	2.866	.114	1.571
c^kc^k	8	6.8	.502	.069	.110	.039	10	.949	.055	.474
c^kc^d	1	7.0	.611	....	.134	.134	6	1.031	.150	.521
c^kc^r	1	4.0	.183	....	.040	.040	13	.616	.048	.284
c^kc^a	4	4.0	.513	.039	.047	.023	4	.489	.025	.211
c^dc^d	21	6.8	.676	.129	.148	.032	23	1.150	.082	.589
c^dc^r	10	4.6	.365	.118	.080	.025	19	.593	.046	.271
c^dc^a	1	4.0	.279	....	.061	.061	18	.484	.036	.208
c^rc^r	1	0.0	.054	....	.012	.012	2	.133	.017	.007
c^rc^a	2	0.0	.058	....	.009	.009	..	.....	....	.....
c^ac^a	1	0.0	.058	....	.013	.013	..	.....	....	.....
	eeff						*eeff*			
C–	11	6.8	.636	.138	.139	.042	7	.888	.053	.439
c^kc^k	2	1.0	.057	.011	.012	.009	..	.....	....	.....
c^dc^d	10	2.7	.150	.051	.033	±.010	10	.280	±.031	.092

* Total includes genotypes in which the second allele was unknown.

left from the experiments were examined and compared with the standard pelts. Although it is difficult to evaluate small wisps of hair, there was no doubt whatever that all of the samples of grade 12 and three of grade 11 had faded noticeably. These observations, therefore, indicate that the lighter intensities of adult animals are foreshadowed in some cases, at least, by a fading of the color as early as three to four weeks of age.

It will be noted that in harmony with the previous observations of

RUSSELL, the steps from 0–12 can be regarded approximately as percentage rather than absolute increments. A transformation comparable to hers was therefore made by plotting the logarithms of the corrected colorimetric means (experimental determinations minus .057, the value for white) against the corresponding grades and passing a line through the points by the method of least squares. The weights (W) were inversely

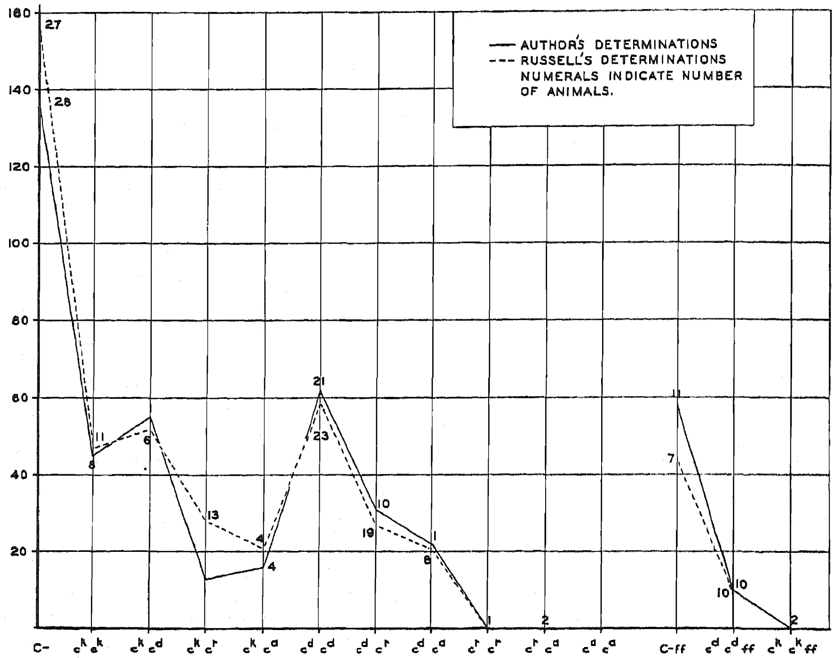

FIGURE 1.—A comparison of the corrected colorimetric values for yellow genotypes with those estimated by RUSSELL. Scales are made roughly comparable by multiplying RUSSELL's corrected values by .572.

proportional to the estimated squared standard errors on the logarithmic scale. Since small deviations (δCV) on the original scale become $\dfrac{\delta \mathrm{CV} \log_{10}{}^{e}}{\mathrm{CV}-.057}$ on the logarithmic scale, the standard error on the latter was taken as $\dfrac{.434\ \mathrm{SE}}{\mathrm{CV}-.057}$. The standard error of the slope $\dfrac{\Sigma \mathrm{WGY}-\bar{\mathrm{Y}}\Sigma \mathrm{WG}}{\Sigma \mathrm{WG}^2-\bar{\mathrm{G}}\Sigma \mathrm{WG}}$ was taken as $\dfrac{.434}{\sqrt{\Sigma \mathrm{WG}^2-\bar{\mathrm{G}}\Sigma \mathrm{WG}}}$. The close agreement between the author's results and those of RUSSELL is revealed by the essential identity of the slopes in the following equations

$$\log (CV-.057)=\bar{8}.735+.138G \text{ (not weighted)}$$
$$\log (CV-.057)=\bar{8}.812+.132G\pm.0039 \text{ (weighted)}$$

and those quoted by RUSSELL

$$\log (CV-.12)=\bar{8}.987+.136G \text{ (not weighted)}$$
$$\log (CV-.12)=\bar{9}.032+.135G\pm.0046 \text{ (weighted).}$$

If RUSSELL's line be made to intersect the author's at grade 7, her equation becomes

$$\log (CV-.12)=\bar{8}.779+.135G$$

It is obvious that neither weighting nor the use of a series of standards in place of the single one of grade 7 used by Russell has resulted in any substantial differences. Slightly significant departures from linearity (weighted line) are indicated by t values of −2.9, −3.1, +3.0, −2.7, −2.8 for grades 3, 4, 6, 11, 12 respectively, and also by grouping of the signs for t in grades 1–4, 5–10, 11–12.

The tabulations according to genotype (table 2 and figure 1) are of interest in evaluating the dominance relations of the *C* series. In order to make the scales of RUSSELL and the author comparable, the corrected values as given by her must be multiplied by .572, the factor which was found to make her value for grade 7 as given by the line coincide with that of the author. Table 3 gives the percentage of the intensity of type

TABLE 3

Percentage of the intensity of C—F— in other gene combinations.

GENOTYPE	PRESENT DATA		RUSSELL'S DATA	
	NO. OF ANIMALS	PERCENT	NO. OF ANIMALS	PERCENT
$C-F-$	28	100.0	27	100.0
c^kc^kF-	8	32.0	11	30.2
c^kc^dF-	1	39.8	6	33.2
c^kc^rF-	1	9.0	13	18.1
c^kc^aF-	4	11.2	4	13.6
c^dc^dF-	21	44.4	23	37.5
c^dc^rF-	10	22.2	19	17.3
c^dc^aF-	1	15.9	8	13.2
$c^{ra}c^{ra}F-$*	4	0.0	2	0.0
$C-ff$	11	41.6	7	27.9
c^kc^kff	2	0.0	0	.....
c^dc^dff	10	6.7	10	5.9

* The double superscripts used throughout this paper signify all of the possible gene combinations indicated by the letters. For example, $eec^{ra}c^{ra}F-=eec^rc^rF-$, eec^rc^aF-, eec^ac^aF-, all phenotypically white; $e\,e\,c^d\,c^{ra}ff=e\,e\,c^d\,c^rff$, $e\,e\,c^d\,c^aff$, phenotypically white; $e\,e\,c^d\,c^{ra}\,F-=e\,e\,c^d\,c^r\,F-$, $e\,e\,c^d\,c^a\,F-$, phenotypically cream.

($C-F-$) found in other combinations (after subtraction of the correction factor .057).

The author's determinations are in essential agreement with the previous observations of RUSSELL and also with expectations based on WRIGHT's grades. The evidence indicates that C is dominant over its lower alleles, that $c^k c^k$ and $c^d c^d$ reduce the intensity markedly (to 32 and 44 percent of $C-F-$, respectively, after subtraction of the correction factor .057), that with $c^r c^r$ and $c^a c^a$ the threshold for the production of yellow has not been attained. The readings for $c^k c^k$, $c^k c^d$, $c^d c^d$ agree with all previous

TABLE 4

A comparison of the colorimetric and permanganate numbers (PN) for sepias tabulated by grade.

	PRESENT DATA					RUSSELL'S DATA		
GRADE	NO. OF ANIMALS	MEAN CV	σ	GENERALIZED σ	S.E.	NO. OF ANIMALS	MEAN* PN	S.E.
21	18	1.440	.212	.214	±.050	9	144	± 7
20	13	1.297	.176	.192	.053	5	115	7
19	17	1.011	.159	.150	.036	6	120	7
18	10	.894	.134	.133	.042	1	103	15
17	9	.755	.163	.112	.037	2	96	10
16	4	.782	.066	.116	.058	1	99	15
15	7	.609	.059	.090	.034	6	75	5
14	6	.526	.101	.078	.032	2	64	6
13	2	.478	.021	.071	.051	3	56	6
12	1	.347	....	.052	.052	2	62	7
11	2	.330	.039	.049	.035	..	...	..
10	1	.390	....	.058	±.058	..	...	±..

* PN is the abbreviation for "permanganate number" as defined by RUSSELL

$$(PN) = cc\ KMnO_4 \times NKMnO_4 \times 100/\text{grams hair}.$$

The values given in the table are uncorrected. To secure the corrected mean in each case subtract 12, the estimated value for adsorbed impurities.

results in falling within a relatively narrow range (weighted averages: present data: 40.9 percent of $C-F-$, RUSSELL's data 34.8 percent). There are, however, differences: $c^d c^d$ is significantly more intense than $c^k c^k$ (t=4.7). Similarly $c^k c^r$, $c^k c^a$, $c^d c^r$, $c^d c^a$ fall within narrow limits, but at a level somewhat less than half that of the preceding genotypes (weighted average: present data 45.3 percent of $c^{kd} c^{kd}$, RUSSELL's data 47.1 percent). Again the combinations involving c^d are significantly more intense than those involving c^k (t=4.0). The apparently greater intensity of c^k combinations is contrary to the slightly lower intensities observed by WRIGHT in the stocks on which he published in 1927. Further study will be necessary to determine how far these differences are due to real variations in

the effects of the genes and how far to differences in associated minor factors.

The drastic effect of ff is revealed by readings from $e\,e\,C-F-$, $e\,e\,C-ff$; $e\,e\,c^dc^d\,F-$, $e\,e\,c^d\,c^d\,ff$. In the presence of C, ff has about 42 percent (present data) or 28 percent (RUSSELL's data) as much pigment as F. In the presence of $c^d\,c^d$, ff has about 15 percent (present data) or 16 percent

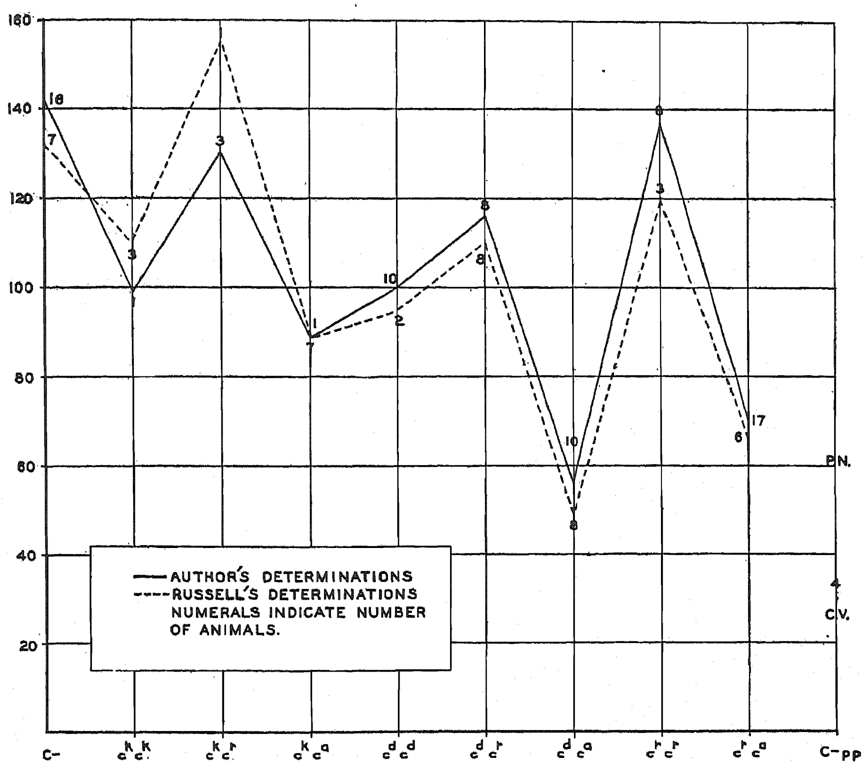

FIGURE 2.—A comparison of the colorimetric values and permanganate numbers for sepia genotypes. Scales are made comparable by multiplying the corrected permanganate numbers by .0101.

(RUSSELL's data) as much pigment as F. The disproportionately larger effect in $c^d\,c^d$ foreshadows the complete elimination of yellow in $c^d\,c^{ra}\,ff$ in contrast with the considerable quantity present in $c^d\,c^{ra}\,F-$.

The sepia series

The data for the sepia series are given by grade in table 4 and for genotype in table 5 and figure 2. The same procedure was followed in calculating the standard errors as that previously described for the yellow series. Since there are probably real differences in the coefficients of variability of genotypes (for example, $c^r\,c^a$ extending over 7–10 grades in comparison

to $c^k c^{ka}$ which cover only four grades, WRIGHT 1925) it is probably not as legitimate to generalize the standard deviations of genotypes as grades. In all cases standard deviations calculated in the usual manner are included together with the generalized standard deviations.

The determinations by grade show a consistent drop from grade to grade except for 16 and 10 in which the numbers were small. As with the grades

TABLE 5

A comparison of the colorimetric and permanganate number (PN) for sepias tabulated by genotype.

	PRESENT DATA						RUSSELL'S DATA			
	$E-P-$ DARK-EYED SEPIAS						$E-P-$ DARK-EYED SEPIAS			
C SERIES	NO. OF ANIMALS	MEAN GRADE	MEAN CV	σ	GENERALIZED	S.E.	NO. OF ANIMALS	MEAN* PN	S.E.	TRANSFORMED PN
CC	1	19.00	1.329	....	.233	±.233	..	...	±..	.....
Cc^d	5	20.80	1.358	.221	.238	.076	..	...	..	.....
Cc^a	10	21.00	1.561	.171	.274	.123	..	...	..	.....
$C-$	16	20.75	1.420	.216	.249	.062	7	143	9	1.323
c^kc^k	1	21.00	.994	....	.174	.174	3	120	12	1.091
c^kc^r	3	20.00	1.297	.250	.228	.131	1	166	26	1.555
c^kc^a	7	18.86	.885	.098	.155	.059	1	100	17	.889
c^dc^d	10	18.40	.996	.160	.175	.055	2	106	13	.949
c^dc^r	8	19.25	1.164	.284	.204	.072	8	121	7	1.101
c^dc^a	10	14.20	.562	.106	.099	.060	8	60	4	.485
c^rc^r	8	20.00	1.368	.194	.240	.084	3	131	12	1.202
c^rc^a	17	16.18	.699	.156	.123	.030	6	77	6	.657
	$E-pp$ PINK-EYED SEPIAS						$E-pp$ PINK-EYED SEPIAS			
$C-$	4	11.00	.349	.036	.060	±.030	1	76	±14	.646

* The values given are uncorrected. To secure the corrected mean in each case subtract 12, the estimated value for adsorbed impurities.

of yellow, it is also obvious here that the absolute decrements are large at the upper end of the scale and comparatively small at the lower end. The same methods were therefore applied in calculating the line which best fits the points determined by the logarithms of the colorimetric values plotted against grade (G). The slopes found for the colorimetric values and RUSSELL's titrations are shown in the following equations:

$\log CV = \bar{8}.893 + .060G$ (unweighted, grades 21–13)

$\log CV = \bar{8}.849 + .062G \pm .0030$ (weighted, grades 21–13)

$\log CV = \bar{8}.865 + .061G \pm .0025$ (weighted, grades 21–10)

$\log (PN - 12) = .997 + .053G \pm .0048$ (weighted, grades 21–13)

$\log (PN - 12) = .953 + .056G \pm .0025$ (weighted, grades 21–2).

The line given by RUSSELL's weighted equation for grades 21–13 was transformed so as to intersect the author's line at grade 17. RUSSELL's line then became:

$$\log (PN-.12) = \bar{9}.002 + .053G.$$

Evidently the use of weights makes substantially no difference to the slopes in the first two equations. RUSSELL's data contain no determinations for grades 10 and 11 and only two for grade 12. Thus the most suitable equations for comparison are those for grades 21–13, although the

TABLE 6

Percentage of the intensity of C—P— in other gene combinations.

GENOTYPE	PRESENT DATA		RUSSELL'S DATA	
	NO. OF ANIMALS	PERCENT	NO. OF ANIMALS	PERCENT
$C-P-$	16	100.0	7	100.0
c^kc^kP-	1	70.0	3	82.5
c^kc^rP-	3	91.3	1	117.5
c^kc^aP-	7	62.3	1	67.2
c^dc^dP-	10	70.1	2	71.7
c^dc^rP-	8	82.0	8	83.2
c^dc^aP-	10	39.6	8	36.7
c^rc^rP-	8	96.3	3	90.9
c^rc^aP-	17	49.2	6	49.6
$C-pp$	4	24.6	1	49.7

line for the colorimetric values is sufficiently stable so that inclusion of grades 10, 11, 12 does not alter the slope appreciably. The difference in slope constants .062 and .053 for grades 21–13 as given by the colorimetric and permanganate methods cannot be regarded as significant ($t = 1.6$). The colorimetric equation shows no significant departure from linearity since a comparison of calculated and observed values gives estimates of 2.4 or less for t and the signs are not clustered. There is, of course, no equation which includes colorimetric readings for grades 2–12 to compare with RUSSELL's in as much as the colorimetric method was not applicable to grades 2–9.

The close agreement between the permanganate and colorimetric values, especially in genotypes in which the numbers were fairly large, is shown in tables 5 and 6 and figure 2. The last column in table 5 gives the values of RUSSELL, transformed by multiplying each of her entries (corrected) by .0101, the factor found to make the lines of RUSSELL and the author coincide at grade 17. From tables 4, 5 and 6 and figure 2 we see evidence for the complete dominance of C over its lower alleles, a rather high degree of dominance of c^k over c^a ($c^k\,c^a = .89 c^k\,c^k$) and approximate intermediacy of $c^d\,c^a$ and $c^r\,c^a$ compared to $c^d\,c^d$ and $c^r\,c^r$ ($c^d\,c^a = .56\ c^d\,c^d$, $c^r\,c^a = .51\ c^r\,c^r$).

Determinations for $c^d c^r$ are elevated when compared to $c^d c^d$ as are also $c^k c^r$ when compared to $c^k c^k$, $c^r c^r$ compared to $c^d c^d$, and $c^r c^a$ compared to $c^d c^a$ (within the limits indicated by the standard errors). These data are confirmatory of previous observations made by WRIGHT on the basis of grade alone and also of RUSSELL's results. The most serious discrepancy between the permanganate and colorimetric values is in the pink-eyed sepias of constitution $C-$ in which the ratios of $C-P-$ to $C-pp$ are approximately 2 and 4 respectively. The numbers are small, one and four, but the colorimetric value would appear to give the more reliable estimate as judged by the relatively small standard deviation and the appearance of the hair as determined by the grades assigned.

SOURCES OF VARIABILITY

The data indicate a rather wide variability within both grade and genotype. At first sight one might expect such fluctuations within genotype on the basis of a consideration of the range of grade within genotypes (WRIGHT 1927), but comparatively small differences within grades since all animals of a given grade look alike. A careful examination of the hair samples, however, indicates differences in the distribution of pigment from tip to base within grade. These differences are less developed in the short hair at birth (when the grades were assigned) than at three to four weeks. No attempt has been made to take the basal color into account in grading. Causes for this variation in gradient are as yet unanalyzed although there is evidence that modifying genetic factors play a role. In addition to this source of variability, subjective errors of grading are occasionally responsible for differences of a grade.

The reasons for the instability of readings within genotype are not wholly known, but some suggestions as to the sources of variation can be given. The non-isogeneity of the hereditary background certainly suggests one possible factor; the role of environmental agents is also of probable importance. There is no doubt about the presence of at least one modifying genetic factor which reduces the intensity of both yellow and sepia (unpublished data from a stock not included in the present investigation but derived from the lighter sepias and yellows of the stock used). The large amount of literature on temperature and plucking with its consequent cooling effect shows that the deposit of pigment may be modified by these factors. The fact that the standard pelts undergo some fading of color with time and exposure to light while being used points to light as another controlling source of variability. Competition for food within the cages may also help to account for differences within litter-mates of the same genetic constitution. Here it is appropriate to note the works of HARTWELL (1923) and HAYDAK (1935) which have shown that the intensity of the color in

black rats could be reduced by restrictions of diet. The intensity could be restored in part at least by resumption of the stock diets or others containing adequate sources of proteins such as tyrosine and tryptophane, and other food constituents. A consideration of all of the above factors leaves little wonder that the colorimetric and permanganate readings show comparatively large fluctuations.

SUMMARY

1. Colorimetric determinations of the amount of yellow pigment in the hair of known genotypes of guinea-pig have confirmed and extended the previous evaluations by RUSSELL.

2. A colorimetric method was developed by which it has been possible to secure measurements of sepia solutions for grades 10–21. These include black-eyed sepias and pink-eyed sepias of constitution $C-$. Determinations by this method closely parallel the findings of RUSSELL who used a very different method. (Titration of pigment with potassium permanganate.)

3. An analysis has shown that the total experimental error for the yellow series (one set of ten readings) was 4.2 percent and for the sepia series 2.8 percent. About 18 percent of the total error was due to colorimetry in the case of the yellow series, and 22 percent in the sepia series.

4. The colorimetric determinations indicate that C is completely dominant over its lower alleles in both the yellow and sepia series, and that $c^k\,c^a$, $c^k\,c^r$; $c^d\,c^a$, $c^d\,c^r$ compounds in the yellow series show somewhat less than half as much pigment as $c^k\,c^k$ and $c^d\,c^d$ respectively. Genotypes $c^r\,c^r$, $c^r\,c^a$, $c^a\,c^a$ are pure white in place of yellow. The drastic reduction of yellow by ff is shown to be disproportionately larger in $c^d\,c^d$ than in $C-$. In the sepia series, c^k shows a fairly high degree of dominance over c^a, but little or no dominance is exhibited by c^d and c^r over c^a($c^a\,c^a$, white). All comparisons show that c^r produces more sepia than c^d. Genotypes of $C-P-$ constitution gave readings which were roughly four times those of $C-p\,p$.

5. The determinations for grade and genotype in both series showed considerable variability. Suggestions as to factors which may control these fluctuations are given.

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ON THE IDENTIFICATION OF SEGREGATED PHENOTYPES IN PROGENY FROM CREEPER FOWL MATINGS

DOROTHEA RUDNICK AND VIKTOR HAMBURGER

Storrs Agricultural Experiment Station, Storrs, Connecticut, and Washington University, St. Louis, Missouri

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IN 1930 LANDAUER and DUNN established that the genetic basis of the Creeper character in fowl is a single Mendelian factor pair, lethal in homozygous condition. The heterozygotes are viable and show the characteristic chondrodystrophy that has earned the designation 'Creeper.' The homozygotes as a rule show marked pathological changes on the third day of incubation and die shortly thereafter; occasionally they survive until near hatching time, in which case they are strikingly phokomelic (LANDAUER 1933). The present study has two general purposes: 1) to examine the time of phenogenetic segregation of the various genotypes from Creeper matings, since this information is fundamental to a study of the action of the *Cp*-factor; 2) to give practical indications for early identification of these genotypes, as a necessary basis for any experimental work on early stages.

LANDAUER (1932) has shown that at 72 hours' incubation approximately 25 percent of the embryos from a Creeper × Creeper mating ($Cp + \times Cp +$) are markedly retarded in body size and differentiation. He has further shown that at 48 and even at 36 hours' incubation approximately one-fourth of the embryos fall into a developmentally retarded group—as regards both somite number and dimensions—a group almost discontinuous from the main population (table 5). The obvious inference is that it is this retarded group that represents the homozygous lethal segregates, and this has until now been the basis for early identification of these segregates.

In order to establish this criterion on a firm experimental basis, and to find if it holds for stages earlier than 36 hours' incubation, we undertook independently a series of experiments in which somites were counted at definite periods of incubation, and the subsequent development of the individual embryos observed. All embryos came from the same stock, which has been maintained at Storrs; $Cp + \times Cp +$ matings were used, as well as one mating of $Cp +$ hens with a $++$ cock. Experiments were carried on at Storrs and at St. Louis. At Storrs a large forced-draft incubator was used, maintained at 36 degrees centigrade with very slight fluctuations. At St. Louis, a Buffalo incubator, without forced draft, was used at 38–39 degrees, with some slight fluctuations above and below this range.

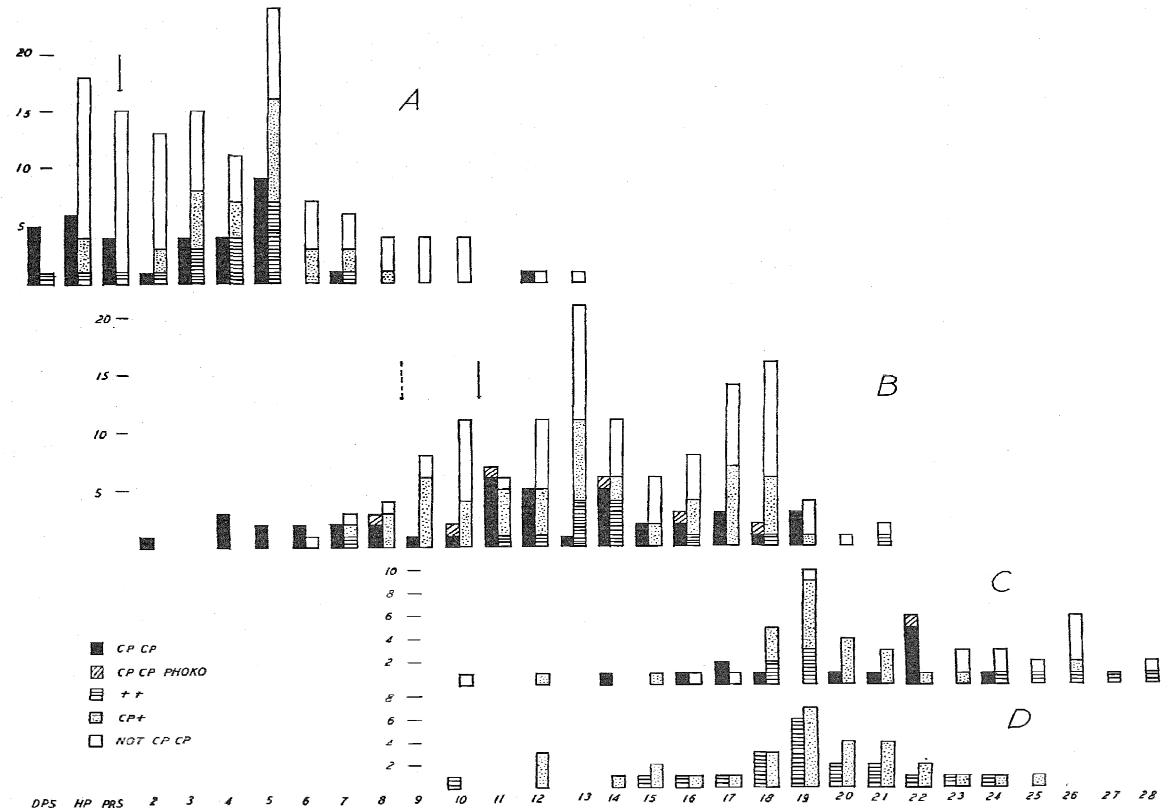

FIGURE 1.—Block diagrams showing distribution of somite stages among the segregates of $CP + \times CP +$ matings (A, B, C) and $CP + \times + +$ (D); A at 24–25 hours' incubation, B at 32–44 hours, C at 48–50 hours and D at 48 hours. DPS = Definitive primitive streak stage; HP = head-process; Pr S. = pre-somite; other stages defined by somite number.

For the somite counts, a window was made in the egg and the blastoderm was stained lightly with neutral red transferred from an impregnated agar plate. The somites were counted under a binocular microscope, and the egg closed and allowed to incubate further. Instruments and materials were of course sterilized. Final observations were made either at 3–4 days, which permits distinction between pathological and viable embryos, or at 8–10 days, which permits recognition of phokomelic, heterozygous and normal types respectively. The operative procedure clearly does not interfere with subsequent expression of these various characters. Only embryos showing the typical symptoms—early pathology or later phokomelia—were classified as *Cp Cp*; only typical chondrodystrophics as *Cp* +; doubtful cases have been eliminated from our tables.

RESULTS

Figure 1 is a block diagram summarizing the somite development of the various segregates at three stages of incubation. The first three distributions are for embryos from *Cp* + × *Cp* + matings. The first one (A) consists of embryos of 24–25 hours' incubation. The second one (B) groups cases examined at 32–44 hours' incubation. It was found that the range of somite variability in 36 hour embryos operated at various times in the spring and early summer was so great that additional cases both earlier and later than this standard stage could be added without increasing the spread. The third distribution (C) is of cases of 48–50 hours' incubation. Developmental stages are listed on the abscissae, number of cases on the ordinates, and at each stage two columns are shown, one of *Cp Cp* types (that is, early pathological and phokomelic forms) and a second including all viable segregates—*Cp* +, normal, and viable embryos opened too early to be classified. This latter category may include a small number of phokomelic homozygotes which look normal at 3–4 days.

Discrimination between Cp Cp and all other groups

a. Somite counts. In the 24–25 hour cases, the means have been calculated by simply assigning a number to each stage, beginning with the definitive primitive streak as stage 1, and later converting the mean back to the corresponding somite stage for the sake of uniformity. This procedure may be open to criticism as unduly distorting time-relations, but it must be recalled that the somite stages themselves are far from being evenly spaced on the developmental time-scale: to this unevenness we attribute, for example, the excessively large number of 5 somite stages. The means given here must be considered quite strictly as means of stages, not of time-classes. When calculated this way, the mean for *Cp Cp* embryos at 24–25 hours is 2.4 somites ± 2.8 and that for all non-*Cp Cp* em-

bryos is 3.7 somites ± 2.9. Hence the mean for homozygotes is slightly but far from significantly lower than that of normal embryos. The variance is much the same. There is then no natural break in distribution between the two groups.

The 32–44 hour cases show a similar result. Again the mean for *Cp Cp* embryos is lower (11.6 ± 4.5 somites) than that of non-*Cp Cp* ones (13.8 ± 3.4 somites) but the difference is clearly not significant. In this case the variance of the *Cp Cp* embryos is greater than that of the normal ones.

In both of the foregoing distributions it can be seen that the lower somite brackets have more than the expected 1:3 ratio of *Cp Cp* embryos. In the 48–50 hour group even this is not true. The *Cp Cp* mean is only slightly lower than that of the non-*Cp Cp* embryos (19.9 ± 2.9 as against 21.0 ± 4.0) and the variance is less, so that several cases of very low somite number are found to be non-*Cp Cp* embryos.

b. Other morphological symptoms. At this latter stage of incubation (48 hours and more) the morphological symptoms of the lethal effect begin to be observable; in a few of our cases we could predict from the first examination that an embryo would be of the *Cp Cp* type.

LANDAUER (1932, tables 7, 8) has described and analyzed by measurements the head-retardation in 72 hour *Cp Cp* embryos, and has shown that the posterior part of the body is less inhibited than is the head (his tables 1, 2). He has also described the abnormalities of the heart and circulatory system as well as of the limb regions. We have made some additional observations on the development of the lethal syndrome that may be of interest here.

Normal embryos undergo drastic morphogenetic changes in the 17–20 somite period (about 48 hours' incubation). The cephalic flexure increases from a slight bend to an angle of 90 and more degrees, and the forebrain elongates concurrently. Meanwhile the whole head rotates to lie on its left side, and the amnion closes over it. The vitelline arteries commence to consolidate, and rapidly become large conspicuous trunks. The heart becomes a loop. These changes show a certain independence of one another and of somite formation. For example, an embryo (from a normal mating) may have a completely rotated head without the amnion's covering it completely. Vitelline artery function may begin at any stage of this phase of head-morphogenesis and between the 18–20 somite stages. The establishment of the vitelline arteries is of course a conspicuous step in the transformation of the area vasculosa to a functional circulatory network, arising from the union of discrete blood islands.

The deficiencies of the *Cp Cp* embryos are primarily these: the head region begins showing retardation at the 17–20 somite stage; its flexure and rotation are delayed, although they eventually proceed, the flexure to at

least 90 degrees, the rotation to completion. The head does not grow in volume at anything comparable to the normal rate; by 72 hours' incubation microcephaly is striking. (See LANDAUER 1932, Plate XI figures 2–4; Plate XII figures 1, 8). Sections through the head at this stage show extreme thinning of the brain wall—sometimes to only two cell layers—as well as marked cellular degeneration in the nervous system. The head mesenchyme is also very deficient in quantity in the examples we have examined histologically.

Accompanying the diminished head growth is usually a striking asymmetry expressed in the eyes and the otocysts. At the time of rotation, the left side, that is, the side on which the embryo comes to lie, begins to be differentially retarded. The retardation may even precede rotation: in one case, a 20 somite embryo which had not yet undergone rotation and which showed other symptoms of the lethal effect, the left optic vesicle was seen to be slightly delayed as compared with the right, in making contact with the lateral head ectoderm. Following this stage, in *Cp Cp* embryos, the right eye continues development at about the same pace as the rest of the head, forming a small eye-cup and lens. The left eye, by 72 hours, usually has barely started to form a cup, and the lens remains a thickening continuous with the body wall, occupying a groove in the optic vesicle. In fixed preparations such lenses are apt to be everted and to project outside the body; this may be only an artifact due to the abnormal fluid pressures in the embryo. In the living state these left eyes appear as very small shrunken knots of tissue. The left otocyst may be similarly delayed in closure and retarded in growth.

The heart, as LANDAUER has pointed out, shows much variability, being retarded in form in a degree corresponding to the head retardation, but often being quite enlarged in size as compared to the normal heart of a similar morphological stage. This variability extends to the heart wall as well. Some cases we have examined histologically show a fairly good amount of myocardial tissue; others show absolutely no thickening even in the ventricle.

The thinning of tissues that is marked in the head-region at 60 or 72 hours is apparent also in the trunk, although so much more variable that posterior cross-sections in some examples look quite normal. The posterior neural tube is not necessarily thinned or degenerating. The somites are usually very deficient in cell mass; the process of segmentation itself is very little retarded by comparison, as LANDAUER shows and as the present results also indicate. The Wolffian ridge remains a thin layer, filled with mesenchyme, instead of being thickly packed with cells. Small limb buds may appear, delayed with reference to the rest of the body form.

While these events are occurring in the embryo, similar failures appear

in the vascular area. Although some attempt is made at forming vitelline arteries, and a feeble circulation may always be observed in the main channels, the peripheral blood islands never become incorporated in the network and no effective vitelline circulation is ever formed. The vascular area remains full of discrete blood clots, and the whole appearance becomes more and more anaemic as the blastoderm expands on the third and fourth days. In sections it is seen that endothelium is differentiated from the blood islands throughout the vascular area, but that these either do not interconnect, or connect only by very fine openings, so that the erythroblasts are not free to move, but remain in densely packed masses. Within the embryo too the circulation is defective; extravasation and clotting occur in the main channels, which are greatly distended; in sections various non-vascular cavities such as the amniotic space and the coelom are found full of erythrocytes.

During the fourth and fifth days of incubation, thinning and degeneration of tissues continues and fluid spaces enlarge progressively; the embryo becomes vesicular and shapeless. Hearts were found still beating as late as the fifth day. Complete necrosis probably ensues before the seventh day.

All these changes are foreshadowed in embryos of 48 or more hours which have reached the 20–21 somite stage without establishment of strong vitelline arteries. A few of our cases in the 48–50 hour group, as we have said, were such embryos; they subsequently developed the typical pathology. However, all *Cp Cp* embryos are not distinguishable at this stage, as can be judged from two cases of 22-somite embryos in which vitelline arteries were observed to be functioning. These two were subsequently found to be *Cp Cp* individuals. Hence the stage when all *Cp Cp* embryos are distinctly diagnosable must be put at the 24–25 somite period (48–54 hours' incubation). Lack of strong functional vitelline arteries is unmistakable at this stage of somite development, as is head retardation.

Discrimination between heterozygous and normal embryos

Figure 1 also presents somite stages for Creeper and normal embryos. Here the distribution is clearly parallel. In the 24–25 hour lot, the *Cp* + mean is 4.1 somites ± 2.0; that for normals is 3.6 somites ± 1.9. In the 32–44 hour group, the *Cp* + mean is 13.1 ± 3.4; the normal mean 13.8 ± 3.1. In the 48–50 hour group the *Cp* + mean is 19.5 ± 2.7 as against 22.3 ± 3.8 for the ++ embryos. The fourth distribution (D) on the table is the somite record for *Cp* + and ++ progeny in a mating of Creeper hens by a normal cock, from which the two sorts of offspring are expected in equal quantities. This series, incidentally, was made at approximately the same season (May-June) as most of the preceding 48–50 hour group, and it is

obvious that the spread of stages is approximately the same. In this lot the mean of the *Cp* + embryos is 19.9±2.9 somites, that of ++, 21.0±4.0 somites. There is possibly a slight tendency for the normal embryos to be more advanced than the heterozygous, but it is obvious that none of these differences in any way approaches significance.

To illustrate the reliability of the general table as indicating variability actually realized in small lots of eggs, we include summaries of two individual experiments. One (Storrs: May) consisted of 19 usable cases, examined at 24 hours. Of the 7 later found to be *Cp Cp*, one was in the definitive primitive streak stage, one pre-somite, one 2-, one 4- and three 5-somites respectively. One 5 somite embryo was found to be normal at 72 hours. *Cp* + embryos were found at 2, 3, 5, 6 somites, ++ ones at 3, 4 and 5 somites. The other experiment (St. Louis: February) was at 40 hours' incubation. *Cp Cp* embryos had 8 and 11 somites; one 9 somite embryo was found to be not *Cp Cp*; four 9 somite cases and one of 10 somites turned out to be *Cp* +. Thus in individual settings, as well as in the total population, *Cp Cp* embryos may be distributed throughout the whole range.

DISCUSSION

We conclude from the evidence presented above that in the stages and under the conditions employed, *heterozygous and normal* segregates from Creeper matings are not distinguishable on the basis of somite formation nor by other morphological criteria. Distinct macroscopic differences appear not earlier than on the 7th day (LANDAUER 1931).

Concerning the early segregation of *Cp Cp*-embryos and their viable sibs, LANDAUER (1932) states: 1) that homozygous embryos are smaller than normal ones, at least from the 36th hour of incubation on; 2) that "with regard to the number of somites the homozygous Creeper embryos lag most conspicuously behind the normal ones during the period when in normal development a rapid multiplication in somite number takes place (close of the 2nd day)" (p. 391). No striking difference in somite number was found at 72 hours of incubation.

Our data refer to somite numbers only. They show a slight shift toward the lower somite groups at 24–36 hours; this difference disappears at 48 hours. Thus we extend the continuous grouping of somite number shown by LANDAUER (1932, table 5) for 72 hour embryos back to the 48 hour stage, and we find that the artificial grouping in his 48 hour series is not in agreement with the constitution of the embryos. At 36 hours this grouping is not as strict a break as he suggests, but a statistical trend; the same is true at the 24 hour stage.

We have not collected data on size differences within our material. Some

of LANDAUER's data on size differences may require revision in view of the present findings. The 36 and 48 hour embryos classified as "homozygous" (table 4) are identified as such by the criterion of somite number only and may therefore include a higher percentage of "normal" embryos than is assumed by the author (p. 368).

The size differences found by him at these stages are probably only the expression of differences in developmental stage, superimposed of course on great individual variability. If, for example, we examine head length (the dimension showing greatest reduction in the retarded individuals: p. 374) in those embryos of the 36 hour group which had 12 somites and were classified as normal (table 4, column 2, last 6 cases), we find a variation of from 1.60 to 1.83 mm, with a mean of 1.71 ± 0.09. In the 48 hour embryos classified as *Cp Cp*—that is, those with 12–15 somites—the head length varies from 1.20 to 2.04 mm with a mean at 1.49 mm ± 0.07. The probability that these groups belong to the same normal distribution is between .4 and .3 (FISHER 1936, p. 128 and table 4), and hence the difference of the means cannot be taken as statistically significant. There is no demonstration in this material—even though a number of individual cases in the tables may suggest it—that 12–15 somite embryos of 48 hours' incubation are absolutely or differentially smaller than embryos that have taken only 36 hours to reach the same stage.

A reinvestigation of the matter of size difference of the various genotypes as contrasted with developmental stage would be especially desirable since LANDAUER's interpretation of the mode of action of the Creeper-factor as a primary growth retardation is partly based on these data on size differences in early *Cp Cp*-embryos. His data on 72 hour embryos (table 1 and 2) are not in doubt; however, they do not concern the essential point: whether or not growth differences *precede* the pathological abnormalities—both of which are strikingly apparent at 72 hours' incubation. This question cannot be considered as definitely settled until the search for micro-pathological changes has been extended to stages preceding the 17–20 somite stages using material of which the genetic constitution is checked.

For the practical problem of selecting *Cp Cp* embryos in early stages, counts of somite numbers are not reliable as far as the detecting of individual cases is concerned. Selecting in the lowest quarter of the population (the expected proportion of *Cp Cp*) in no instance gives even a 50–50 chance of obtaining a homozygote. From the table it can be seen that in the lowest one-fourth of the 24–25 hour group (marked off by the solid arrow) we have only about a 1:2 chance of selecting a *Cp Cp*. In the 32–44 hour group, the lowest one-fourth gives a similar ratio (16:27); if we select in the lowest one-eighth (to the left of the dotted arrow) the ratio rises to 13:8; embryos below 6 somites are all *Cp Cp*. This suggests that in a group

of approximately 36 hour embryos, averaging 13–14 somites, embryos that are greatly retarded (less than 6 somites) will probably be *Cp Cp*. In the 48–50 hour group, where one begins to find other morphological criteria of retardation, somite number ceases to be a differential in any sense.

As an example of the effect of selection, we may cite the ratios obtained by us individually. The expectation in the population is of course 1:3 for *Cp Cp*: non-*Cp Cp*; and 2:1 for *Cp* +:+ + among those surviving till 8–9 days. The Storrs material (D. R.) was taken without any selection whatever. The 32–50 hour St. Louis material (V. H.) was partly used for other experiments and a small fraction of the highest somite groups discarded. The ratios in the various groups follow:

	STORRS				ST. LOUIS			
	Cp Cp: non-*Cp Cp*		*Cp* + : + +		*Cp Cp*:non-*Cp Cp*		*Cp* + : + +	
24–25 hours	27	76	28	19	8*	48*	—	—
32–44 hours	23	65	19	10	25	61	30	4
48–50 hours	9	32	11	8	5	12	10	2
Cp+×++ 48 hours	—	—	30	21	—	—	—	—

* We are indebted to Mr. J. Cairns for placing these data at our disposal.

Thus selection of low somite embryos in practice selects against non-*Cp Cp* and evidently for homozygous embryos.

Differences in head length are useless for practical purposes; they are too slight in view of the great variability in length and developmental stage in a given age group. The only reliable criteria are the pathological symptoms listed above, particularly the asymmetry in eye and otocyst and the failure to establish a proper vitelline circulation. They become apparent at the end of the second day but show a certain variation in the time of their first manifestation.

SUMMARY

Embryos from Creeper×Creeper (*Cp* +×*Cp* +) matings were examined for somite number at three stages of incubation: 24–25 hours, 32–44 hours, 48–50 hours. Some cases from a *Cp* +×+ + mating were also used at 48 hours. They were incubated farther and their phenotypic fate ascertained. The following conclusions are drawn:

1. There is no basis in somite number or morphology for distinction between the + + and *Cp* + segregates in these stages.

2. At the two earlier stages (24–25 hour, 32–44 hour) the *Cp Cp* segregates are on the average slightly less advanced in somite development than are their viable sibs, the mean somite number being 2.4 as compared with 3.7 at 24 hours; 11.6 as compared with 13.8 in the 32–44 hour group.

The distributions are entirely continuous, as is shown in figure 1. Selection in the lower quarter of these groups would give better than a 1:3 chance of obtaining a *Cp Cp* individual.

3. At 48–50 hours, somite number ceases to be a differential between the *Cp Cp* and the viable segregates. At this time, the first signs of pathological changes appear in the *Cp Cp* individuals. Some observations on the course of these pathological changes are offered in supplement of the descriptions of LANDAUER (1932).

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THE INTERRELATIONS OF TEMPERATURE, BODY SIZE, AND CHARACTER EXPRESSION IN DROSOPHILA MELANOGASTER[1]

JAMES V. NEEL
The University of Rochester, Rochester, New York

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INTRODUCTION

DURING the past half-century, numerous investigations have shown that the exact expression of many wild-type and mutant characteristics of diverse organisms depends upon the temperature at which development takes place. Concurrently, studies on relative growth have frequently demonstrated a regular relation between the size of the whole individual and the degree of development of certain of its organs. Between these two fields of investigation, which have grown up quite independently, there is a bridge. It is supplied by the fact that in some organisms, such as the insects, adult size is related to the temperature prevailing during development. Consequently, the results of any study of the effects of temperature upon character expression in these forms are possibly complicated by the failure of size to remain constant at all temperatures.

The present paper constitutes a step in an analysis of the interrelations of temperature, body size, and character expression in an insect, *Drosophila melanogaster*. It will be shown that in the cases of polychaetoid and Dichaete, two "temperature-responsive" mutants of this species, a large part of the apparent "temperature effect" is under the conditions of these experiments bound up with changes in body size attendant upon the temperature change. Were the flies of the various temperature series of equal size, then the so-called "temperature effect" would be greatly reduced.

The principal conclusions of this paper were summarily reported at the Seventh International Genetics Congress.

THE POLYCHAETOID MUTANT

Flies homozygous for this mutant (*pyd*, $3\text{–}39 \pm 2$) are characterized by the presence of extra bristles at or near the various normal bristle loci. Supernumerary macrochaetae are particularly likely to occur in the dorsocentral and scutellar regions. A detailed description of the mutant will be given by NEEL (1940). The stock employed in this investigation had been inbred in small mass cultures (three-four pair matings) for two years prior to the beginning of the experiments.

[1] This and a following paper (Genetics, in press) have been submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at the University of Rochester.

The size of the fly and the expression of polychaetoid, at constant temperature

The relation between the size of *pyd* flies and the number of bristles present has been investigated in several different ways. Thus, in a first approach to the problem, groups of *pyd* flies were raised under such unlike nutritional conditions as to result in differences in average fly size from group to group, and the relation between mean size, as expressed by body weight, and the mean number of dorsocentral bristles present per side per fly (M dc) established. As a standard procedure, two or three pairs of flies were introduced into half-pint milk bottles containing approximately 50 cc of corn-meal agar and allowed to remain there seven to eight days. The bottles were kept in an incubator similar to the two-shelf type described by BRIDGES (1932), in which temperature stayed within 0.2° of 24.0°C. When the offspring began to emerge the flies were collected at three day intervals. From one to four collections were made from a set of bottles, and thus groups of flies developing at different nutritional levels of the cultures and hence of different mean sizes were secured. The flies of each collection period were given unlimited access to food during the intervals between collections. Immediately after collection, all the males were etherized and weighed together, and the average body weight established. Only males were used in the weight determinations, since their weight is less subject to fluctuation than that of the females. For all groups the time elapsing between removal from food, etherization, and weighing was made as short as possible (ca 10–15 minutes). This minimized errors due to dehydration of the flies. The M dc was then calculated. A total of ten groups of flies, representing a range in average fly weight of from 0.600 ± 0.011 to 0.953 ± 0.004 mg, and in M dc of from 2.322 ± 0.052 to 3.470 ± 0.034, was thus secured. The mean number of males weighed in a group was 98.7; an average of 139.6 half thoraces from these were used in the M dc determinations. The complete data for this experiment and for the similar experiments to be reported below have been filed with GENETICS.

A simple expression for the relation between average body weight and M dc was desired. It was found that while a plot of M dc against average body weight yielded an approximation to a straight line, a better approach to linearity was secured with a double log plot (figure 1). This indicates that the data can be treated as an expression of the power function

$$y = bx^k$$

recognized by biologists as the relative growth function. In this case, y corresponds to M dc, x to mean body weight, and b and k are constants.

The ranges of the variables x and y are towards the lower limits of valid applications of logs.

The line which has been fitted to the points of figure 1 is the regression line of log M dc on log mean body weight, as determined by the least squares method with unweighted ordinates. The equation of this line is

$$Y = 0.713X + 0.577$$

where Y corresponds to log M dc and X to log mean body weight. The aberrant point seen in the lower left hand corner of the figure has not been

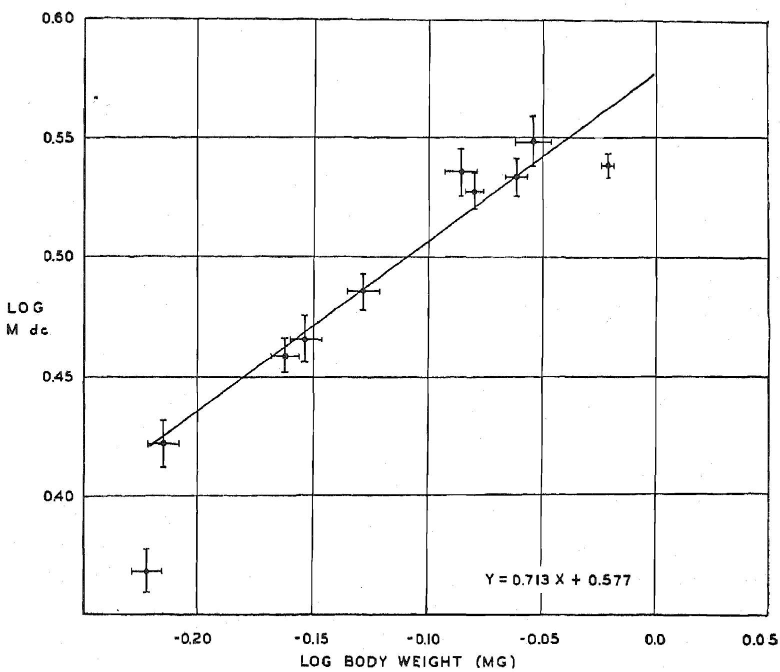


FIGURE 1.—The correlation, in groups of male *pyd* flies raised at 24.0°C at various nutritional levels, between log average body weight and log M dc. The vertical lines attached to each point represent the limits of $\pm\sigma_m$ for the M dc. The horizontal bars indicate the calculated magnitude of $\pm\sigma_m$ for the average weight. All logs are to the base 10. Details in text.

included in the fitting of the line, since for reasons unknown it is in such obvious disagreement with the others. The flies from which the extreme right hand point in the figure was derived were raised under somewhat different conditions than the rest, namely, from eggs laid over a two to six hour period and allowed to develop in finger bowls of 10.5 cm diameter containing approximately a one centimeter layer of agar, one hundred to a bowl.

Each of the points shown in figure 1 is based upon two means, M dc and mean body weight. The determination of these means involves both methodological and sampling errors. In consequence of the simplicity of the operations involved (counting bristles and weighing groups of ca 50–150 flies on a Becker Chainomatic Balance with a sensitivity of 0.2 mg), methodological errors are small. Sampling errors are of somewhat greater magnitude. The sampling error for bristle number is given by the standard error of the M dc (σ_m). The upper and lower limits of the vertical bar attached to each point of figure 1 are respectively M dc$+\sigma_m$ and M dc$-\sigma_m$.

The evaluation of the sampling error for weight, which is the standard error of the mean body weight of a group, is somewhat more difficult. In the course of the experiments reported in this paper, two different methods have been used in raising groups of flies of different average sizes. a) The one method, used in securing the flies incorporated into figure 1, involved the withdrawal from culture bottles of flies emerging during successive three day periods, and has already been described in some detail. b) The other method, used in the derivation of the points of figures 3, 4, 5, and 6, was to place varying numbers of eggs from two to eight hour egg-laying periods on a given quantity of food (amount contained in a one centimeter layer of corn-meal agar in a finger bowl of 10.5 cm diameter). It seems that the first method should lead to a somewhat greater variation in body weight within the group than the second. Accordingly, the means of estimating the weight error which follows is based upon flies raised by the first procedure; this should be adequate for flies raised by the second method.

One hundred male flies composing a portion of the first three days emergence from culture bottles subjected to standard conditions were weighed individually on a microbalance with a sensitivity of 0.001 mg. Repeated weighings of the same fly showed that the accuracy of the weight determination was within one percent. For these 100 flies, the mean body weight (M) was 0.881 mg; the standard deviation (σ) was 0.089 mg; the standard error of the mean (σ_m), 0.009 mg; and the coefficient of variability (CV), 10.1. From these determinations, an estimate of the error of the weight determination for each group can be made. It is assumed that from group to group, no matter what the average body weight, CV remains approximately constant and equal to the value found in these 100 flies. Hence in any other group, σ_m may be reasonably estimated as equal to .01 $M/\sqrt{N}$, since $\sigma_m = \sigma/\sqrt{N} = (CV \times M)/100\sqrt{N}$. The standard errors so estimated are indicated in figure 1 by horizontal bars.

Some of the points in figure 1 deviate significantly from the fitted line, where the criterion of significance is deviation by an amount equal to three

times the standard error of body weight or M dc. Similar deviations will be noted in later figures. Possible causes for these deviations are as follows: 1) The relation between the two sets of values studied may not be strictly linear. Thus, HUXLEY (1932) has pointed out that in cases of insect heterogony, at large body sizes points usually fall below the straight line fitted to a double log plot of the data. A deviation which may be of this nature is shown in the figure. 2) Experience has shown that the weight of a given group of flies may from time to time vary considerably, depending on feeding conditions. In this work, an attempt was made to give each group of flies the same free access to food, prior to weighing. It is possible that this attempt was not entirely successful, and that therein lies the cause of at least part of these deviations.

A second determination of the relation between size and dorsocentral number consisted in deriving the correlation in individual flies between femur length and total number of dorsocentrals present. Femur length has been used as an index to body size by a number of investigators (cf BREHME 1939), but the exact relation between these two variables has not been established. Since an investigation of this point seemed desirable, the right fore-femurs of the males composing each of the ten samples incorporated into figure 1 were mounted, and the mean femur length of each sample determined from camera lucida drawings of the mounted femurs. Over a range in mean femur length from 516.0 ± 4.55 to $582.0 \pm 3.16\mu$ and in mean body weight from 0.600 ± 0.011 to 0.953 ± 0.004 mg, these two variables appeared to be linearly related. It seems probable that with uniform age and feeding this group relation would hold good for the individual flies which compose the group.

Male flies were collected at intervals over a period of three weeks from cultures kept at 24.0°C, in order to get a considerable distribution in size. The right fore-femur and the dorsum of the thorax of each fly considered were mounted together on a slide. For this population ($N = 134$), the correlation between femur length and total number of dorsocentral bristles present on both sides of the fly was 0.619 ± 0.053. There was a still higher higher correlation between femur length and the total length of the dorsocentrals present (0.705 ± 0.044). To determine this latter measurement the bristles were drawn by camera lucida (see NEEL 1940). It seems probable that slight errors of measurement tend to obscure an even greater natural correlation between these variables. A similar strong correlation between the size of individual flies (as measured by over-all length) and dorsocentral number, in an extra-bristled stock, has been reported by MACDOWELL (1915).

The third method of evaluating the relation between size and bristle number consists in correlating the weight of the individual fly with the

number of dorsocentrals present on both sides of the fly. In the sample of 100 *pyd* males which were weighed individually to determine the standard error of mean body weight (p. 228), the correlation was 0.278 ± 0.092. An insignificantly higher value (0.308 ± 0.090) is obtained when the logs of the two variables are correlated. This correlation between size and bristle number, although significant, is much looser than that indicated by the other two approaches. There are two outstanding reasons for this. 1) The range in body size in these 100 flies was not as great as that utilized in the

TABLE I

The relation between length of egg-larval life and number of dorsocentral bristles present in male pyd flies, when size is held constant.

a. Femur length between 569 and 581μ				
Interval after oviposition at which puparium formation occurred (hours at 21°C)	132.0–139.9	140.0–147.9	148.0–155.9	156.0–163.9
Number of flies	28	35	59	19
Mean total number of dorsocentrals $\pm \sigma_m$	6.71 ± 0.21	6.68 ± 0.15	6.68 ± 0.12	6.64*
b. Femur length between 582 and 594μ				
Interval after oviposition at which puparium formation occurred (hours at 21°C)	132.0–139.9	140.0–147.9	148.0–155.9	156.0–163.9
Number of flies	26	77	41	8
Mean total number of dorsocentrals $\pm \sigma_m$	7.11 ± 0.21	6.72 ± 0.11	7.00 ± 0.12	6.75*

* No σ_m calculated because of the small number of flies involved.

other two experiments. Since a correlation between two variables becomes more apparent as the range of either is extended, the smaller size distribution probably contributes to the relatively low correlation observed. 2) The flies weighed were those emerging in the first three days from a set of bottles subject to conditions described above. The weighings required two days. Although every effort was made to give the flies unlimited access to food, microscopic investigation at the time of weighing revealed considerable differences from fly to fly in the degree of distension of the abdomen. These are in part due to age differences. Because of this variation in abdominal contents, body weight must be regarded as a less accurate index of innate fly size than femur length.

In a group of flies, random weight fluctuations from fly to fly would be expected to cancel out, so that average body weight for the group, as compared with that for other groups, would probably be an accurate index of

mean size. This appears a reasonable explanation for the fact that for different groups of flies the correlation between average body weight and M dc is quite high (figure 1), although for individual flies the correlation between weight and total number of dorsocentrals is much lower.

It is apparent from each of these three procedures that at a constant temperature bristle number is linked with body size; an estimate of the closeness of the association depends upon the particular approach used.

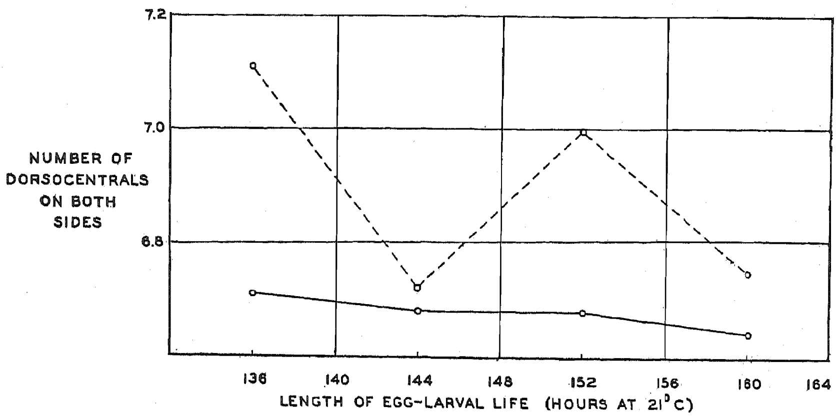


FIGURE 2.—The lack of correlation of length of egg-larval life with the total number of dorsocentral bristles present in *pyd* flies. The circles connected by the broken lines represent groups of flies with femurs 582–594μ long, while those connected by solid lines are based on flies with femurs 569–581μ in length. Details in text.

Independence of M dc and length of larval life, at constant temperature

Groups of flies of different average sizes have been obtained by varying cultural conditions. Thus, some of the groups used in deriving the regression equations developed at quite low nutritional levels. A concomitant of a restriction on food intake such as practiced here is an increase in the average duration of larval life. It seemed necessary to test for a possible correlation of this prolongation of the larval stadium with bristle number, for the existence of such a relation would complicate any interpretation of the size-bristle number relation. Accordingly, an experiment was designed which would permit a comparison of the total number of dorsocentrals in equal-sized flies whose larval lives had been of unequal lengths.

Eggs laid by *pyd* females over a two-hour period were transferred to finger bowls containing yeasted corn-meal agar; each bowl received sufficient eggs that the resulting larvae developed under sub-optimum food conditions. This insured a considerable distribution in the time of puparium formation. Development took place at 21°C. The great majority

of puparia were formed from 132 to 164 hours after oviposition. New puparia were isolated every four hours during this period. When the flies emerged, the males of each collection period were classified as to the total number of dorsocentrals present and the right fore-femur length. A total of 527 flies was treated in this manner. Some males with femurs between 569 and 594μ in length were found in each collection. The mean total number of dorsocentrals of flies having femurs 569 to 581μ long was calculated for each four-hour group. A similar calculation was made for flies whose femurs ranged in length between 582 and 594μ. Since this subdivision of the data meant that the number of cases in any four-hour group was small, adjacent groups have been combined, so that there are available four eight-hour periods.

The data are given in table 1. In figure 2 for each of the two femur length ranges (= fly sizes) mean number of dorsocentrals has been plotted against length of larval life. For each eight-hour group, the length of larval life has been treated as the mid-point of that period. The broken line connects circles representing groups of flies with femurs 582–594μ long; the circles connected by the solid lines are based on flies with femurs 569–581μ in length. No significant trend in the data is evident. This suggests that the inequalities in length of larval life observed at the various nutritional levels are of no significance in the determination of bristle number.

The relation between temperature and M dc

To test the effect of temperature upon fly size and M dc, well fed *pyd* females were allowed to oviposit at room temperature on food exposed in small rectangular metal containers. The eggs obtained in a two to eight-hour egg-laying period were divided into lots of 100 and placed on well

TABLE 2

The effect of temperature upon bristle number and body weight in pyd Drosophila melanogaster males. M dc stands for the mean number of dorsocentrals present per side per fly.

TEMPERATURE (°C)	M dc $\pm\sigma_m$	NO. HALF THORACES	AVERAGE BODY WEIGHT (MG $\pm\sigma_m$)	NO. FLIES WEIGHED
14.0°	4.533 ± 0.033	542	1.061 ± 0.006	276
19.0°	4.213 ± 0.023	754	1.062 ± 0.005	441
24.0°	3.470 ± 0.034	536	0.953 ± 0.004	500
29.0°	3.058 ± 0.022	740	0.784 ± 0.003	396

yeasted corn-meal agar contained in finger bowls of 10.5 cm diameter. The bowls were then put at the various experimental temperatures. Such a procedure should insure each larva a plentiful supply of food. Subsequent to puparium formation, the puparia were placed in vials furnished with enough food that the flies could feed freely after emergence.

The temperatures at which the flies were raised and the M dc and average fly weight at each temperature are given in table 2. A 15°C increase in temperature is associated with a pronounced decrease in the M dc, from 4.533 ± 0.033 at 14°C to 3.058 ± 0.022 at 29°C. Simultaneously, however, mean weight is decreasing. In view of the above-demonstrated correlation between fly size and M dc, a question immediately arises. To what extent is this effect of temperature upon bristle number concerned with some bristle-forming process within the body not related to fly size, and to what extent is the effect mediated either through size or some processes common to size and M dc?

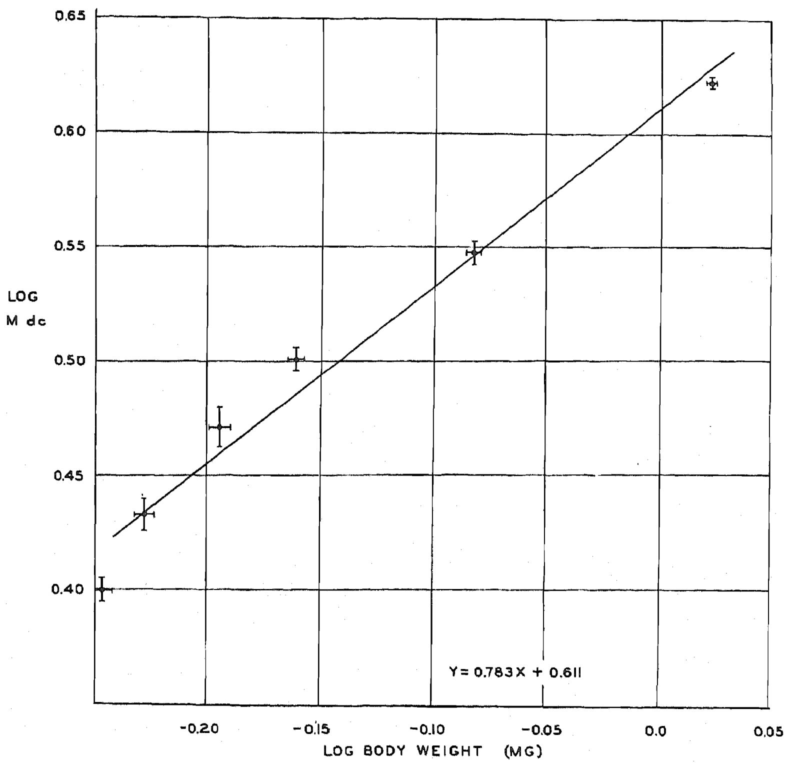


FIGURE 3.—The relation, in groups of *pyd* flies raised at 19.0°C at various nutritional levels, between log average body weight and log M dc.

An evaluation of the manner of action of temperature

Given that there is a change in the M dc from one temperature to another, then let a direct effect of temperature upon bristle number be defined as one which is independent of the size differences at the various temperatures, while an indirect effect is one which under these experimental conditions is bound up with a size change. The question raised in

the last paragraph may be restated thus: what are the relative roles of these two effects in the total temperature effect?

To answer this question, equations for the regression of M dc on weight at each of the three lower temperatures employed are necessary. This equation has already been obtained at 24.0°C (p. 227). The relation at the other two temperatures was determined by placing egg samples from two to eight-hour laying periods under various food conditions (different numbers of eggs per finger bowl containing food). Groups of flies showing wide differences in average body weight were secured.

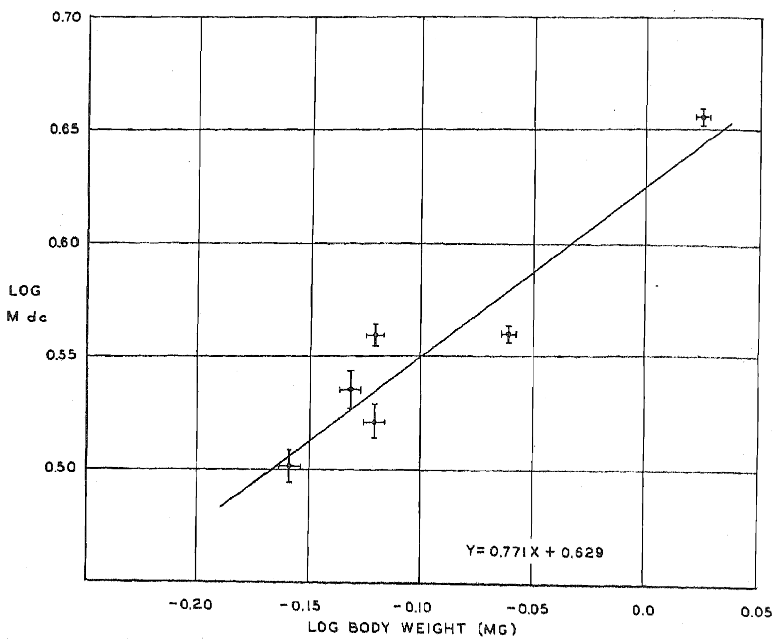


FIGURE 4.—The correlation between log average body weight and log M dc, in groups of *pyd* flies raised at 14.0°C at different nutritional levels.

Six lots of flies were raised at 19.0°C. The highest mean body weight secured for any group was 1.062 ± 0.005 mg; this was accompanied by an M dc of 4.213 ± 0.023. These were the flies which have already been discussed, raised under optimum conditions and listed in table 2. The lowest mean body weight was 0.568 ± 0.005 mg; the corresponding M dc was 2.508 ± 0.035. In agreement with the 24.0°C data, a working approximation to a linear relation is observed when log M dc is plotted against log average body weight (figure 3). The equation for the regression of log M dc on log weight is

$$Y = 0.783X + 0.611.$$

Six groups of flies, varying in weight from 1.061 ± 0.006 to 0.694 ± 0.007 mg and in M dc from 4.533 ± 0.033 to 3.175 ± 0.049, were raised at 14.0°C. The distribution of the data is such that it would be difficult to determine the exact nature of the regression. However, on the assumption that it is not different from that believed to obtain at 24.0° and 19.0°C, a straight line of best fit to a double log plot of the results has been obtained (figure 4.) Its equation is

$$Y = 0.771X + 0.629.$$

Possible causes for the significant departure of some points from the fitted line have already been discussed.

These regression equations make possible a determination of the relative importance of direct and indirect temperature effects. Two methods of evaluation have been employed. The first is summarized in table 3. In

TABLE 3

An evaluation of the relative importance of direct and indirect temperature effects in the expression of the pyd mutant of Drosophila melanogaster.

A	B	C	D	E	F	G	H
TEMPERATURE RANGE	OBSERVED M dc AT LOWER TEMPERATURE	OBSERVED M dc AT HIGHER TEMPERATURE	DIFFERENCE B−C	CALCULATED M dc AT LOWER TEMPERATURE*	DIFFERENCE B−E (=INDIRECT TEMPERATURE EFFECT)	DIFFERENCE D−F (=DIRECT TEMPERATURE EFFECT)	RATIO OF F/G
29°–24°	3.470	3.058	0.412	3.177	0.293	0.119	2.46
29°–19°	4.213	3.058	1.155	3.373	0.840	0.315	2.66
29°–14°	4.533	3.058	1.475	3.532	1.001	0.474	2.11
24°–19°	4.213	3.470	0.743	3.936	0.277	0.466	0.59
24°–14°	4.533	3.470	1.063	4.102	0.431	0.632	0.68
19°–14°	4.533	4.213	0.320	4.457	0.076	0.244	0.31

* If size equals maximum size at higher temperature.

column A of the table the six temperature combinations possible from these data have been listed. Column B shows the M dc at the lower of the two temperatures composing a given combination, while column C gives the M dc at the upper of the two. Column D, showing the difference B − C, gives the total temperature effect on the M dc over the given temperature range. From the regression equations which have been developed, it has been calculated what the M dc would be at each of the lower temperatures, if average fly size were equal to that obtaining at the higher temperatures. The results of these calculations are entered in column E. The difference between the entries in columns B and E, given in column F, is the change in M dc correlated under these conditions with the size change

(indirect effect). The difference between the entries in columns D and F (or E−C), given in column G, is the change in M dc independent of a size factor (direct effect). Finally, the ratio of the entry in column F to that in G (column H) is the ratio of indirect to direct temperature effects over the given range. It appears that under these experimental conditions the major portion of the effect of temperature upon the M dc is indirect. The role of the indirect effect is not the same at all temperatures, but is greatest at high temperatures (where the M dc is changing most rapidly) and least at low.

In the above procedure, in columns B and C have been listed the actually observed M dc at the observed maximum size. This M dc does not always correspond to that expected on the basis of the regression equation. This is of course due to the circumstance that not all points fall right on the regression line. In this evaluation it is possible to use, not the actual observed M dc at maximum size, but the M dc which as calculated from the appropriate regression equation should accompany this size. This modification of the procedure results in a slightly higher average value for the ratio of indirect to direct effects. If the deviation below the line of the terminal points in the 24.0° and 19.0°C results is a systematic rather than a random occurrence, then this modification is less valid than the original procedure.

A second approach to this evaluation problem has been developed by DR. D. R. CHARLES of this laboratory, who has kindly given me permission to incorporate the method and its results into this paper.

EIGENBRODT (1930) has shown that as temperature decreases, weight increases, down to ca 17°C; "below 17°C there is very little increase in weight" (p. 395). Similarly, RIEDEL (1934) has found that the tibia length of wild-type flies increases with temperature decrease until the 15–16°C point is reached. With further reduction in temperature, tibia length now decreases. The data contained in the present paper (see also below) tend to confirm these observations, that in the neighborhood of 15°C fly size is at a maximum. On the assumption that weight is an exponential function of temperature deviation from 15°C, the following equation has been found to describe satisfactorily the observed relation of temperature to body weight, under optimum larval feeding conditions:

$$\log W = 0.0373 - 0.0007\,|T - 15|^{2.041}. \qquad (1)$$

In this equation, W corresponds to body weight and T to temperature.

The M dc is a function of both size and temperature. The equation derived from the preceding data which relates these three variables is:

$$\log B = 0.640 + 0.800 \log W - 0.0069[T - 15]^{0.990}. \qquad (2)$$

Here W and T have the same significance as in (1), and B represents M dc.

Equations (1) and (2) may be written as

$$W = 1.0898\ e^{-0.0015|T-15|^{2.041}} \tag{3}$$

and

$$B = 4.365\ W^{0.800}e^{-0.0159\,[T-15]^{0.990}}. \tag{4}$$

For convenience in the following discussion, these equations will be represented by

$$W = k_1\ e^{k_2|T-15|^{k_3}} \tag{5}$$

and

$$B = k_4\ W^{k_5}e^{k_6[T-15]^{k_7}}. \tag{6}$$

From equation (5) it follows that at any given temperature, the rate of change in body weight is given by

$$\frac{dW}{dT} = k_1k_2k_3\,|T - 15\,|^{k_3-1}e^{k_2|T-15|^{k_3}} \tag{7}$$

and the amount of change, ΔW, over a small temperature interval, ΔT, is

$$\Delta W \doteq k_1k_2k_3\,|T - 15\,|^{k_3-1}e^{k_2|T-15|^{k_3}}\Delta T \tag{8}$$

which by substitution from (5) becomes

$$\Delta W \doteq k_2k_3\,|T - 15\,|^{k_3-1}W\Delta T. \tag{9}$$

Equation (6) is of the form $B = f(W, T)$. As a result of partial differentiation of this equation with respect to W, it is seen that as temperature changes, at any instant the rate of change in the M dc linked with the size change may be expressed as

$$\frac{\partial B}{\partial W} = k_4k_5W^{k_5-1}e^{k_6[T-15]^{k_7}} \tag{10}$$

and the amount of change in the M dc linked with the size change (ΔB_W) as

$$\Delta B_W \doteq k_4k_5W^{k_5-1}e^{k_6[T-15]^{k_7}}\Delta W. \tag{11}$$

This by substitution from equation (9) and then (6) and simplification becomes

$$\Delta B_W \doteq k_2k_3k_5\,|T - 15\,|^{k_3-1}B\Delta T. \tag{12}$$

Similarly it is seen that the rate of change in the M dc due directly to the effect of temperature change may be expressed by

$$\frac{\partial B}{\partial T} = k_4k_6k_7W^{k_5}[T - 15]^{k_7-1}e^{k_6[T-15]^{k_7}} \tag{13}$$

while the amount of change in the M dc due directly to the temperature change (ΔB_T) is

$$\Delta B_T \doteq k_4k_6k_7W^{k_5}[T - 15]^{k_7-1}e^{k_6[T-15]^{k_7}}\Delta T. \tag{14}$$

This by substitution from equation (6) becomes

$$\Delta B_T \doteq k_6k_7[T - 15]^{k_7-1}B\Delta T. \tag{15}$$

Over a vanishingly small temperature interval, the ratio of the amount of change due to an indirect effect of temperature to the amount due to a direct effect is

$$\frac{\Delta B_W}{\Delta B_T} = \frac{k_2k_3k_5|T - 15|^{k_3-1}B\Delta T}{k_6k_7[T - 15]^{k_7-1}B\Delta T} = \frac{k_2k_3k_5}{k_6k_7}|T - 15|^{k_3-k_7}. \tag{16}$$

After substitution of the numerical values of the above constants, which are given in equations (3) and (4), equation (16) reduces to

$$\frac{\Delta B_W}{\Delta B_T} = \frac{|T - 15|^{1.05}}{6.25}. \tag{17}$$

In order to evaluate the ratio of indirect to direct temperature effects over an infinitesimal temperature range at any given temperature, it is necessary only to substitute that given temperature for T of the equation. This substitution has been carried out at 1°C temperature intervals over the whole range employed (14.0–29.0°C). The results are given below.

T	14°	15°	16°	17°	18°	19°	20°	21°	22°	23°
$\frac{\Delta B_W}{\Delta B_T}$	0.16	0.0	0.16	0.33	0.51	0.69	0.87	1.05	1.23	1.42

24°	25°	26°	27°	28°	29°
1.61	1.80	1.98	2.17	2.36	2.56

Here again, in agreement with the results given in table 3, it is found that under these conditions the role of the indirect effect is greatest at high temperatures. The chief difference between the results of the two evaluations is that the second method consistently assigns to the indirect effect a smaller value than does the first.

THE DICHAETE MUTANT

These findings concerning the role played by a size factor in the *pyd* temperature effect made it desirable to determine whether similar relationships exist for other temperature-responsive mutants. After a pre-

liminary survey of the literature the *Drosophila melanogaster* mutant Dichaete (*D*, 3-40.4-41.0±) was chosen for an analysis of the type conducted on *pyd*. BRIDGES has shown that this mutant is associated with a short inversion of 3L (MORGAN, BRIDGES, and SCHULTZ 1937). The character is dominant, lethal when homozygous, and in addition to other morphological effects is characterized by a reduction in the number of chaetae present. The dorsocentral bristles are particularly likely to be affected. Either one or both of the two normally present on a side may be missing. When only one is absent, it usually corresponds to the anterior dorsocentral. Occasionally, when one bristle is missing, the remaining does not correspond strictly to either the anterior or posterior dorsocentral, but is located at some point between the usual positions of these two. The character was studied in a balanced Lyra/Dichaete line.

PLUNKETT (1926) found that with an increase in temperature there is a marked decrease in the mean number of posterior dorsocentrals present per side per fly (M p dc) in Dichaete flies. At 24.5°C, where the duration of the egg-larval-pupal period is 9.5 days, temperature was reported to

TABLE 4

The relation of bristle number and body weight to temperature in Dichaete Drosophila melanogaster males. M dc represents the mean number of dorsocentral bristles present per side per fly.

TEMPERATURE (°C)	M dc $\pm\sigma_m$	NO. HALF THORACES	AVERAGE BODY WEIGHT (MG)	NO. FLIES WEIGHED
14.0°	1.530±0.025	268	0.914	137
19.0°	1.557±0.014	788	0.922	417
24.0°	1.356±0.018	480	0.864	243
29.0°	1.213±0.015	632	0.725	325

have an effect upon bristle number from the second to the eighth days. Puparium formation was found to mark the approximate mid-point of the temperature-effective period.

In the present work the criterion of size and temperature effects has been the mean frequency of occurrence of both the anterior and posterior dorsocentrals (M dc), rather than the mean frequency of occurrence of the posterior dorsocentral alone (M p dc) studied by PLUNKETT. This is due to the occasional difficulty, when only one dorsocentral is present, in definitely identifying it as anterior or posterior.

The relation between temperature and M dc

Lyra/Dichaete flies of a long-inbred strain were raised at four different temperatures. The procedure was as described for the similar *pyd* experiment (p. 232). Table 4 shows that the M dc increased from 1.213±0.015 to

1.557 ± 0.014 when the temperature decreased from 29.0° to 19.0°C. However, an additional 5°C decrease, from 19.0° to 14.0°C, was without significant effect upon bristle number. It is of interest to note that the 19.0°C flies are 27 percent heavier than the 29.0°C group, and also slightly heavier than the 14.0°C group. Already in these data there are indications of a parallelism between size and M dc. PLUNKETT (1926) found that in his stock a temperature decrease over the lower part of the range employed did have an effect upon M dc, since the M p dc at 20.0°C was $.439 \pm 0.013$, at 17.0°C 0.581 ± 0.023, and at 14.0°C 0.690 ± 0.036.

The correlation between fly size and Dichaete expression, at constant temperature

Groups of *D* flies were raised at a constant temperature but at different nutritional levels and the relation between M dc and average weight determined. The *D* relation appears to be different from that observed for *pyd*. Whereas in the latter instance an approximation to a straight line was obtained when log M dc was plotted against log average body weight, in the former a straight line relation was observed with a semi-log plot. This is evident from figure 5, where for eight groups of flies of different average sizes raised at 24.0°C, M dc has been plotted against log mean body weight. The equation of the regression line fitted to these points is

$$Y = 3.606X + 1.637$$

where Y corresponds to M dc and X to log mean body weight. The meaning in this apparent difference in the relation of M dc to weight in *pyd* and *D* is not at present clear.

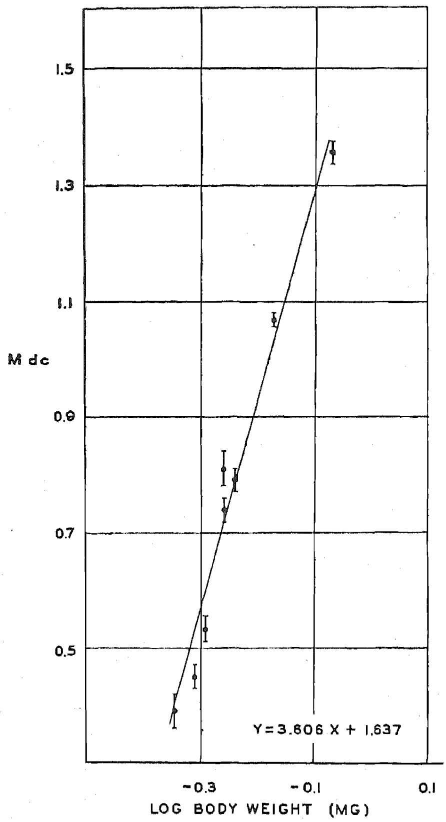
In figure 6 is shown the 19.0°C regression of M dc on body weight. As at 24.0°C, so here an approximation to a straight line is obtained when log weight is plotted against M dc. The equation of the line of best fit to these data is

$$Y = 3.337X + 1.705.$$

The standard error of mean body weight has not been indicated in these figures. The calculation of this constant by the method given earlier demands data on the CV of individual body weight, and such data were not available for Lyra/Dichaete flies.

The flies employed in this work grew very poorly at 14.0°C. Although a number of different egg samples were started under various cultural conditions, only two yielded adults in sufficient numbers to give a reliable M dc and mean body weight. This circumstance makes impossible the derivation of a regression equation at 14.0°C. One of the two groups which did come through at this temperature developed under very favorable

conditions; the M dc and weight for these flies has been given in table 4. By a fortunate coincidence, flies of the other group, grown under less favorable conditions, had an average body weight of 0.726 mg, which is almost identical with the body weight of the 29.0°C flies. The M dc for this group was 1.139 ± 0.021.



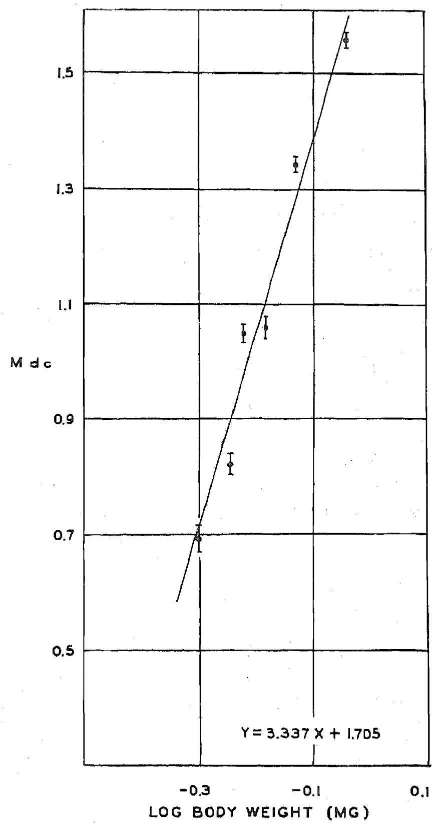


FIGURE 5 (left).—The relation, in *D* fles, between M dc and log average body weight. Each point represents a group of flies raised at a particular nutritional level at 24.0°C. The limits of plus or minus one times the standard error of the M dc are indicated by vertical bars, as before.

FIGURE 6 (right).—As above, but at 19.0°C.

The relation between M dc and length of larval life, at constant temperature

As in the *pyd* case, so for *D* it is necessary to determine whether the increase in length of larval life at lower nutritional levels is correlated with the M dc. PLUNKETT (1926) noted that in *D* flies malnutrition resulted in a decrease in the M p dc, that males have a smaller M p dc than females, and that the introduction of a Minute mutant into a *D* line resulted in a

lowered M p dc. Each of these three factors (malnutrition, maleness, Minutes) was observed to "decrease the size of the flies as well as the rate of development and the mean bristle numbers" (p. 229). Although it was recognized that the data then available were inadequate to analyze the interrelations among these three phenomena, it was thought more probable that the observed decrease in the M p dc was correlated with the increase in the length of developmental time than with the size decrease.

Lyra/Dichaete eggs laid over a two-hour period were placed at 19.0°C under somewhat overcrowded conditions. When puparium formation began, new puparia were isolated every four hours. Each fly after emergence was classified as to femur length and total number of dorsocentrals, and the bristle numbers of equal sized flies compared. The data are given in table 5. Within the limits of this experiment there is no evidence that length of larval life is correlated with the number of dorsocentrals present in *D* flies.

TABLE 5

The absence of correlation between length of egg-larval life and total number of dorsocentral bristles in Dichaete flies, when size is held constant.

INTERVAL AFTER OVIPOSITION AT WHICH PUPARIUM FORMATION OCCURRED (HOURS AT 19°C)	SEX	FEMUR LENGTH	NUMBER OF FLIES	TOTAL NUMBER OF DORSOCENTRALS $\pm\sigma_m$
194.0–210.9	♀♀	580–606μ	28	2.964 ± 0.131
211.0–234.9	♀♀	580–606μ	23	3.087 ± 0.168
194.0–210.9	♂♂	561–587μ	37	2.432 ± 0.096
211.0–234.9	♂♂	561–587μ	26	2.269 ± 0.164

An evaluation of the manner of action of temperature

The roles of indirect and direct temperature effects may now be briefly evaluated (figure 7). The upper, solid line of figure 7 connects circles showing the values obtained for the M dc at each of the four experimental temperatures when fly size was maximum at that temperature. Circles connected by the lower, broken line indicate M dc values to be expected at the same four temperatures if flies were the same (29.0°C) size at all temperatures. The four points connected by this lower, broken line are: 1) The M dc obtained for the 29.0°C flies, 2) the M dc which the regression equation indicates would be found at 24.0°C if average weight were equal to that of the 29.0°C flies, 3) the M dc which from the regression equation would be expected at 19.0°C if average weight were the same as observed at 29.0°C, and 4) the M dc actually obtained at 14.0°C when weight equalled that found for the 29.0°C flies. At 24.0° and 14.0°C this M dc is somewhat

lower than that observed at 29.0°C; at 19.0°C, somewhat higher. In no case is there a wide departure from the 29.0°C value. It cannot be told at present whether the irregularities in the values are of some significance or whether they represent chance deviations from a straight line whose slope may be small and positive, small and negative, or of zero value. Yet it is quite apparent that in *D*, under these experimental conditions the effect of temperature upon the M dc would either be small or non-existent if the flies raised at the different temperatures all had the same average weight.

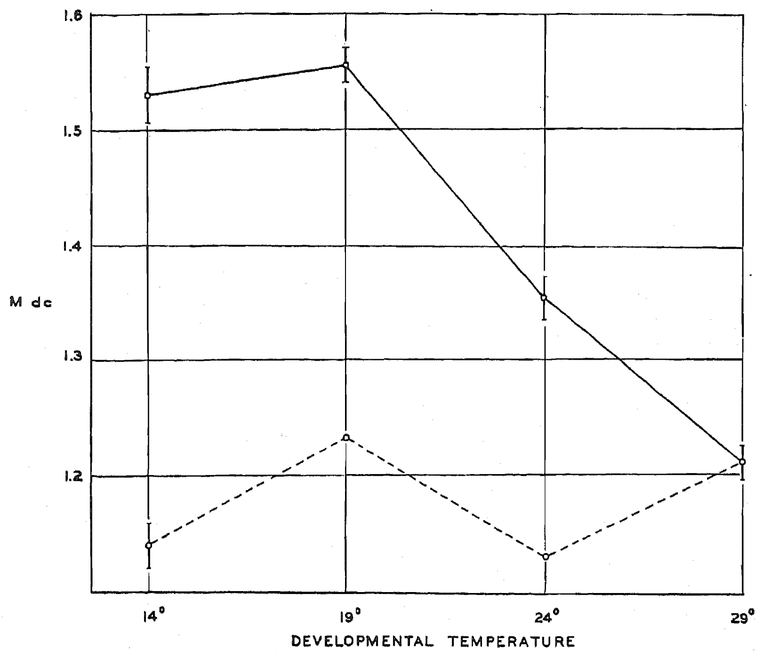


FIGURE 7.—The effect of temperature upon the M dc of *D* flies. The upper, solid-line curve shows the effect of temperature upon the M dc when body size changes at the various temperatures are uncontrolled. The lower, broken line shows the calculated effect of temperature if size were the same at all temperatures. Details in text.

The effect of temperature during the pupal period

PLUNKETT reported that the temperature-effective period in *D* extends over the major portion of larval and pupal life. It was found that when larval life was spent at 24.5°C and pupal at 17.0°C, the M p dc was the same as when larval life passed at 17.0°C and pupal at 24.5°C, that is, "the effect of a higher temperature is the same whether applied to the larval period alone or the pupal period alone" (p. 206). Later work (ROBERTSON 1936, NEEL 1940) has shown that at 24.5°C morphological differentiation

of the bristles in wild-type flies begins early in the second day of pupal development. In view of the difficulty of reconciling this finding with that of PLUNKETT concerning the duration of the temperature-effective period, a repetition of the experiment was undertaken.

Larvae were raised at 19.0° and 29.0°C. When puparium formation began, puparia were collected every four hours. Those formed at 19.0°C were divided into two lots, one of which continued to develop at 19.0°C, and another which completed development at 29.0°C. Similarly, one half of the puparia formed at 29.0°C completed development at this temperature, while the others were placed in the 19.0°C incubator. The results of this

TABLE 6

The effect of temperature during larval and pupal life upon the mean number of dorsocentral bristles per side per fly, in Dichaete males.

LARVAL STAGE AT:	PUPAL STAGE AT:	NUMBER HALF THORACES	M dc $\pm \sigma_m$
19.0°	19.0°	310	1.197 ± 0.019
19.0°	29.0°	206	1.247 ± 0.026
29.0°	19.0°	218	1.000 ± 0.027
29.0°	29.0°	176	0.994 ± 0.030

treatment are shown in table 6. No effect of temperature during the pupal period is evident. This lends further confirmation to the idea of temperature working largely through its effect on size or a factor common to size and M dc. No reason for the discrepancy between these and PLUNKETT'S results can be advanced. (The M dc values given here are considerably lower than those given in table 4 for *D* flies raised under optimum food conditions. This is due to the higher larval densities per unit food in these experiments).

DISCUSSION

Differences in the expression of some characteristic from one temperature to another are frequently treated as due to a direct effect of temperature upon some processes resulting in this characteristic, as these are contrasted with other processes affecting the rest of the imago. A temperature increase is thought of as differentially accelerating some one or few of the many organismic reactions taking place during ontogeny. But temperature, whatever else its effects may be, is a size-altering agent. This fact has frequently been overlooked or disregarded in studies of temperature effects, in spite of a large literature on heterogonic growth. It would therefore seem to be a matter of primary importance, wherever a "temperature effect" is involved, to evaluate in so far as possible the relation of size

changes to this effect. In this paper an attempt has been made to do this for two mutants under certain conditions. The Dichaete data, although presenting certain irregularities commented on above (p. 243), indicate a negligible effect of temperature on mutant expression when there is no size change. In the polychaetoid case, analysis shows that only a minor portion of the temperature effect is due to a direct and differential action of temperature upon some bristle-forming processes, and that the role of this direct effect, relative to the total M dc change, is greatest at the low temperatures.

How general is the situation described for these mutants? In a survey of the Drosophila literature, a number of cases were found where the correlations of both size and temperature with the expression of a given character are known. These cases are summarized in table 7. While the list makes no pretense to completeness, it is probably representative. Some of the characters treated there are wild-type and some mutant. The correlation of decreased size with character expression is not always clearly stated as such in the literature. Thus, it is not uncommon to find references to a "starvation effect" or a "crowding effect" (where excess food is not supplied) in character expression. Obviously in these cases decreased size is involved.

In most of the eleven cases listed in the table we find that the characters studied are oppositely correlated with increased size and with increased temperature (attended as the latter is by a size decrease). The two clear exceptions to the general rule are infra-bar and number of teeth in the wild-type sex-comb.

Is this general agreement in the literature a mere coincidence, or is there a causal relation, namely, is the effect of temperature in part at least through the medium of body size or some factors common to size and the characteristic? Certainly an answer to this question cannot be given at present. For none of the cases listed can conclusions be drawn until it is known what effect temperature has when body size is held constant at the various temperatures, and further, what role increase in length of larval life plays. It is to be expected that the part played in the temperature effect by size factors will be found to vary from mutant to mutant, just as it seems to differ for the *pyd* and *D* cases reported here.

A large amount of work has been done towards determining, for various mutants, the time during development at which temperature exerts its effect upon mutant expression. To cite one example, CHILD (1935b) has reported that in the case of the scute-1 mutant, over a temperature range from 18° to 30° "the temperature-effective period in any one fly lies entirely between the time when 89.3 percent and the time when 96.8 percent

TABLE 7

A comparison of the correlation between character expression and a) increased temperature and b) increased body size. The symbol (+) in column 2 indicates that the expression of the normal or mutant character is better (more pronounced) at a high temperature, while a (−) indicates a poorer manifestation at the high temperature. The symbol (+) in column 4 indicates that the wild-type or mutant character is better expressed at large sizes, while (−) indicates poorer manifestation with increasing size.

CHARACTER	HOW CORRELATED WITH INCREASED TEMPERATURE	INVESTIGATOR	HOW CORRELATED WITH INCREASED SIZE	INVESTIGATOR
wild-type wing length	−	ALPATOV and PEARL (1929), ALPATOV (1930), EIGENBRODT (1930), HERSH and WARD (1932), IMAI (1933), STANLEY (1935)	+	ALPATOV (1930), GAUSE (1931)
fore-femur length	−	ALPATOV and PEARL (1929), IMAI (1932), NEEL (unpub.)	+	NEEL (this paper)
number of teeth in wild-type sex-comb	+	COMBS (1937)	+	CASTLE, CARPENTER, CLARK, MAST, and BARROWS (1906)
Bar series of eye shape alleles				
a. Bar and double-bar	+	SEYSTER (1919), KRAFKA (1920), ZELENY (1923), A. H. HERSH (1924), E. C. DRIVER (1926, 1931)	±	MARGOLIS (1935), BODENSTEIN (1939)
b. infra-bar and double infra-bar	−	LUCE (1926), O. W. DRIVER (1931)	−	LUCE (1931)
vestigial wings	−	ROBERTS (1918), HARNLEY (1930), STANLEY (1931), LI and TSUI (1936), RIEDEL (1937)	+	HARNLEY (1930) CHILD (1939)
scute bristles	some bristles increase in frequency, others decrease	CHILD (1935a)	some bristles increase in frequency, others decrease	CHILD (1936)
the *D. funebris* mutants		NEEL (1937)		NEEL (1937)
ascute	+		−	
evaginated	+		−	
interrupted	+		−	
Missing	+		−	

of the egg-larval development has been completed" (p. 154). Other cases where temperature is reported to have an effect only during a brief portion of the egg-larval-pupal life are numerous.

Available evidence indicates that the effects of temperature on size are distributed over a considerable portion of larval life (IMAI 1937). How is this evidence to be reconciled with the usual relative briefness of the temperature-effective period, if a size factor generally plays a role in temperature effects? As a possible answer to this question, capable of experimental tests, it is suggested that both gross size and the expression of a given character may depend upon some such factor as metabolic level. But whereas size is influenced by the metabolic level of the entire larval period, character expression is primarily dependent upon metabolic level at the time of active differentiation of the character. This time may be only a minor portion of the larval period, and would correspond to the temperature-effective period. Changes in the metabolic level at this time would have a much more profound effect upon character expression than on size.

The present work has shown that under a given set of experimental conditions elimination of size changes is attended by a reduction in temperature effects on bristle numbers. It remains to be determined whether this correlation ever breaks down, and if so, to what extent. From the reasoning of the above paragraph it appears possible that there exist periods in development where a decrease in nutritional level would have an effect upon the expression of a given character out of proportion to the effects on gross size.

At the end of the embryonic determination period of any part, the "size-effective" period for that part must come to an end. Accordingly, the exploration of the role of a general size factor in character expression should involve a measurement of larval or pupal size during and at the end of the embryonic determination of the character. This would not only involve working with larvae, but would necessitate an exact knowledge of the determination period. The assumption is therefore implicit in all this work that adult size is proportional to larval size at the time of determination.

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SUMMARY

1. At a constant temperature the expression of the polychaetoid mutant

of *Drosophila melanogaster* is correlated with fly size. This is shown in three ways:

a. When groups of male flies are raised at varying nutritional levels, a double log plot of the mean number of dorsocentral bristles present per side per fly (M dc) against average body weight yields a straight line.

b. In individual flies, the correlation between femur length and the total number of dorso-centrals present on both sides of the fly or the total length of all dorsocentrals present is high.

c. In individual flies, the correlation between weight and total number of dorsocentrals is significant.

2. Increase in length of larval life is not correlated with the M dc of polychaetoid flies.

3. A decrease from 29.0° to 14.0°C in the temperature at which development proceeds results in an increase in the M dc, from 3.058 ± 0.022 to 4.533 ± 0.033. But at the same time there is an increase in mean weight, from 0.784 ± 0.003 to 1.061 ± 0.006 mg.

4. Equations for the regression of log M dc on log mean body weight at the various experimental temperatures employed are established. From these it is shown that under the present experimental conditions the greatest part of the total M dc change observed over the entire temperature range is correlated with the effect of temperature upon imaginal size.

5. In groups of Dichaete males of different average sizes raised at the same constant temperature, the relation between M dc and log average body weight appears to be linear.

6. No evidence is found for a correlation between length of larval life and the M dc of Dichaete flies.

7. When temperature is decreased from 29.0° to 14.0°C, the M dc increases from 1.213 ± 0.015 to 1.530 ± 0.025. However, over the same temperature range average body weight increases from 0.725 to 0.914 mg.

8. It is shown that if the size of Dichaete flies did not change from one temperature to another, there would be no or only a very slight change in the M dc.

9. The application of these findings in the interpretation of other data concerning the effect of temperature upon character expression is discussed.

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THE PATTERN OF SUPERNUMERARY MACROCHAETAE IN CERTAIN DROSOPHILA MUTANTS

JAMES V. NEEL
The University of Rochester, Rochester, New York

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INTRODUCTION

THE genetical approach to the problem of pattern determination consists in a precise evaluation of the effects of gene substitutions upon a given organismic pattern, and an attempt to explain the results of such substitutions in terms of fundamental processes. If such an approach is to be profitable the pattern studied should be a relatively simple one, and should, further, be one which can be considered in a variety of genetical and environmental backgrounds. Such requirements

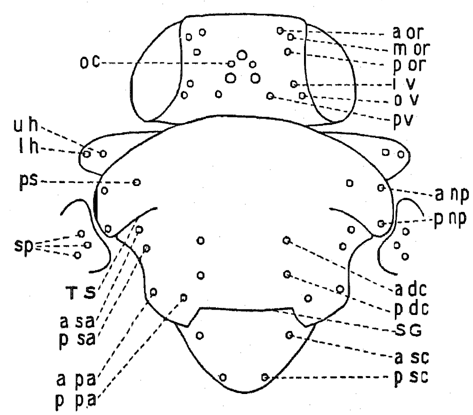


FIGURE 1.—Location and names of the bristles of wild-type *Drosophila melanogaster* and *D. funebris* (after PLUNKETT 1926). Distances on this plane surface are proportional to actual distances on the curved surface of the fly. The abbreviations given in the figure for the various bristles will be employed throughout the text, as follows: a or, m or, p or, anterior, middle, and posterior orbitals; oc, ocellar; i v, o v, inner and outer verticals; pv, postvertical; u h, l h, upper and lower humerals; ps, presutural; a np, p np, anterior and posterior notopleurals; sp, sternopleurals; a sa, p sa, anterior and posterior supra-alars; a dc, p dc, anterior and posterior dorsocentrals; a pa, p pa, anterior and posterior postalars; a sc, p sc, anterior and posterior scutellars; T S, transverse suture, S G , scutellar groove.

are met by the thoracic bristles of Drosophila (figure 1). Each of these is the outgrowth of a single epidermal cell. They all have a characteristic position and size, both of which may be widely altered by various genetic factors.

Studies intended to elucidate the nature of normal and mutant bristle patterns have in the main utilized genes which have as one of their chief

effects the removal of bristles. From the relative frequency of occurrence, in a mutant, of the various bristles which are a constant feature of the normal pattern, and from the associations between the variable bristles of this mutant, conclusions as to the processes underlying certain patterns have been reached. As investigations in point may be cited the work on Dichaete (PLUNKETT 1926) and the studies on the scute series of alleles (DUBININ 1929, STURTEVANT and SCHULTZ 1931, GOLDSCHMIDT 1931, CHILD 1935, IVES 1939).

Only a few pattern studies have involved flies with supernumerary bristles. Chief among these is that of MACDOWELL (1915), who in an investigation of the occurrence of extra bristles in the dorsocentral region was unable to detect any regularity in their distribution. The studies reported in this paper also concerned flies with supernumerary macrochaetae. The use of such flies makes it possible to analyze certain bristle pattern problems which cannot be attacked when bristle-removing genes are employed. In this investigation the emphasis has been not only on the determination of the numbers of bristles present at the various loci and their associations, but more particularly on answering two other questions which have an important bearing on the pattern problem. These are: 1) what is the exact position of extra bristles, and 2) what is their size?

An abstract of this work has already been published (NEEL 1939).

MATERIAL AND METHODS

Five different genotypes have been studied: wild type and *Pch/Pch* of *Drosophila funebris;* wild type, *pyd/pyd*, and *y Hw* dl-49 Theta/*y* sn^3 *bb* of *D. melanogaster*. The three mutant conditions agree in that supernumerary macrochaetae are present on the thorax. Of the various bristle groups affected by the mutants, the dorsocentrals and scutellars can most conveniently be precisely analyzed and so these two groups have been studied chiefly.

All flies were raised at 24.0 ± 0.5°C in half-pint milk bottles, *Drosophila funebris* on banana-agar and *D. melanogaster* on standard cornmeal-molasses-agar. Two or three pairs of flies were introduced into a bottle and allowed to remain there seven to eight days. Only the offspring which appeared during the first three days of emergence was used. All stocks were highly inbred.

Flies of the desired genotype were soaked in saturated KOH for two hours at 60°C. The dorsum of the thorax was then dissected off, dehydrated, cleared, and mounted in balsam under sufficient pressure to flatten it. A camera lucida drawing which included the dorsocentral and, in some cases, scutellar bristles and the scutellar groove was made of each suitable mount. The inclusion of the scutellar groove made it possible to establish

the position of each of the bristles with reference to a definite morphological landmark. There are present on the dorsum of the thorax, exclusive of the scutellum, small microchaetae or hairs; certain of these were included in the drawing. By rolling a small calibrated wheel along the somewhat curved outline of a bristle or hair as drawn, its length in micra could be determined. In order to determine the exact position of the dorsocentrals in wild-type flies, a line was drawn from the base of the a dc to the base of the p dc and extended posteriorly until it intersected the scutellar groove (figure 1). This line will henceforth be referred to as the dorsocentral line. The distance of either the a or p dc from the scutellar groove was determined by measuring along this line from groove to bristle. The same or slightly modified procedures were used in determining bristle positions in mutant patterns.

The need of an abbreviated nomenclature for the dorsocentral bristles when several extra ones are present in the dorsocentral line was met in the following manner. In the normal thorax, where there are two dorsocentrals on a side, the p dc is designated as B1 and the a dc as B2. The distance of B1 from the scutellar groove is L1; the corresponding distance of B2, L2. In a mutant thorax where, for example, there may be four dorsocentrals, the one nearest the scutellar groove is B1 and that most distant, B4. The intervening bristles are B2 and B3. The distances of B1 through B4 from the groove, as measured along the dorsocentral line, are L1 through L4. A similar nomenclature was used for designating the hairs found in the dorsocentral line.

In the determinations of the positions and lengths of these bristles, there are several possible sources of error, notably 1) failure of the thorax, or a bristle on it, to be well flattened on the slide, 2) lack of exact correspondence between the camera lucida drawing and the structure studied, and 3) slight inaccuracies in the measurement of the camera lucida drawing. The total error from these three sources probably does not exceed seven percent for any single measurement; such maximal errors must be rare. Since the figures given in the following tables and diagrams are the mean of a number of flies, and since such errors as do exist are quite comparable and consistent from pattern to pattern analyzed, it seems improbable that inaccuracies of measurement contribute in any way to the differences between patterns to be reported here.

EXPERIMENTAL DATA

The normal bristle pattern in Drosophila funebris

In order to have a standard of comparison with mutant types, as well as to determine the degree of constancy in the size and position of the dorsocentrals in the wild-type, a study was made of 52 half-thoraces from

wild-type *Drosophila funebris* females. The results are summarized in table 1 and graphically shown in figure 2. In figure 2, at the distance from the scutellar groove found to be the mean L1 and L2, heavy lines have been erected the heights of which correspond to the lengths of B1 and B2. The four small, light lines represent the most posterior of the hairs normally present in the dorsocentral line. Morphologically these

TABLE 1

The mean positions and lengths in micra of the dorsocentral bristles and hairs of wild-type Drosophila funebris females.

MEASUREMENT	M	σ_m	σ	MEASUREMENT	M	σ_m	σ
			a. Bristles				
L1	178.8	1.4	9.8	B1	513.4	2.9	20.7
L2	403.4	2.8	19.8	B2	378.2	2.4	17.0
			b. Hairs				
l1	242.9	2.1	15.3	h1	102.9	1.6	11.7
l2	454.0	3.3	23.5	h2	87.1	2.8	20.4
l3	511.0	3.8	27.0	h3	126.7	2.5	18.0
l4	558.7	4.0	28.3	h4	97.5	2.7	19.1

microchaetae differ from the macrochaetae only in size. The figure is in effect a schematic representation of a parasagittal section through the dorsocentral line of a typical, flattened thorax. Each of the mean measurements given in table 1 is not necessarily based upon exactly 52 half-thoraces, since sometimes a slight imperfection in a mounted thorax made it impossible to secure a given measurement. This is also true for the other patterns.

The hairs anterior and lateral to the three figured in front of the a dc have not been studied in detail, but it has been observed that these three are only the first in a row of large hairs extending well forward on the dorsum of the thorax. This is characteristic of the species (STURTEVANT 1921).

From table 1 it can be seen that there is considerable variability in the absolute values of L1 and L2. The outstanding possible causes of this are: a) errors of the method, b) variations in individuals with respect to the size of the thorax, and c) a real variability among equally sized flies with respect to the measurements in question. That size variations in the population are an important contributing factor to the standard deviations obtained can be ruled out. Thus, one would expect that the larger the fly, the further both bristles should be from the scutellar groove and from one another. Therefore, in a population, size variations among individual flies

should establish a significant correlation between the measurements L1 and (L2−L1). Since the correlation between these measurements is actually 0.072±0.137, it is obvious that under the present conditions the position of one bristle is independent of that of the other. Consequently, individual size differences probably do not contribute heavily to the observed standard deviation. The relative importance of experimental errors and positional variability cannot be determined at present.

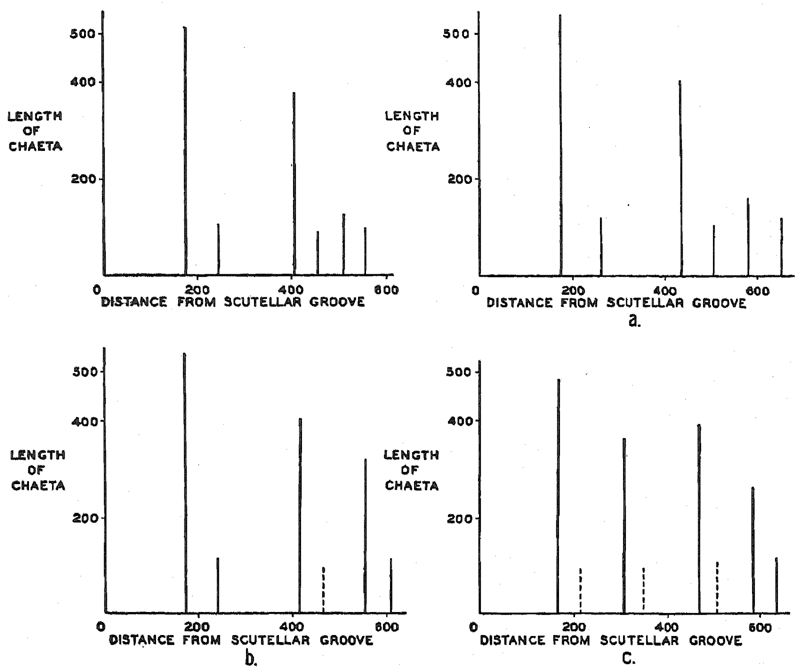


FIGURE 2 (upper left).—A schematic parasagittal section through the dorsocentral line of a typical *Drosophila funebris* wild-type female, showing the distribution and size of the hairs and bristles. For further explanation see text.

FIGURE 3 (upper right, lower left and right).—The distribution and size of the bristles and hairs of the dorsocentral line of *D. funebris Pch/Pch* females, when two, three, or four dorsocentrals are present per side. Where a hair is an inconstant feature of a pattern, it is indicated by a dotted line. Details in text.

A clear tendency for the sizes of the two bristles to vary in the same direction was found; the coefficient of correlation between the lengths of B1 and B2 is 0.578±0.100. What factors contribute to this synchronous variation is not yet evident. The most obvious explanation, that in large flies both bristles are longer than in small ones, is scarcely satisfactory in view of the absence of correlation between L1 and (L2−L1), unless these bristles are much more sensitive to gross size changes than are the thoracic dimensions studied.

Polychaeta in Drosophila funebris

In homozygous condition the autosomal semi-dominant mutant Polychaeta (*Pch*) of *Drosophila funebris* is characterized in the stock employed

TABLE 2

The mean positions and lengths of the dorsocentral bristles and hairs of Pch Drosophila funebris females, when two, three, or four of these bristles are present per side. In no case have the σ and σ_m of a measurement been calculated when the number of cases available is less than twenty.

MEASUREMENT	M	σ_m	σ	MEASUREMENT	M	σ_m	σ
1. The two-bristled pattern							
			a. Bristles				
L1	177.4	1.6	9.4	B1	542.8	3.2	18.4
L2	437.6	2.9	16.8	B2	402.2	3.6	20.6
			b. Hairs				
l1	262.1	3.1	17.5	h1	117.5	2.7	15.4
l2	504.4	3.9	21.1	h2	106.1	6.0	31.6
l3	581.3	4.7	25.6	h3	162.0	5.0	27.4
l4	653.6	4.3	22.5	h4	120.0	4.7	24.7
2. The three-bristled pattern							
			a. Bristles				
L1	174.3	1.4	9.1	B1	538.9	4.9	31.4
L2	415.7	4.2	27.4	B2	402.2	3.9	26.4
L3	553.0	4.7	31.5	B3	315.7	7.1	46.8
			b. Hairs				
l1	241.0	3.1	19.5	h1	118.6	3.3	21.1
l2	463.7	4.9	29.9	h2	97.2	3.4	20.5
		(present in 88.6 percent of the cases)					
l3	610.0	5.8	35.3	h3	114.8	5.5	33.4
3. The four-bristled pattern							
			a. Bristles				
L1	169.4	1.7	8.4	B1	489.2	14.8	71.2
L2	306.7	7.2	34.5	B2	362.2	11.1	52.0
L3	470.8	5.6	26.8	B3	392.1	4.3	20.7
L4	583.3	5.4	25.9	B4	266.7	10.9	45.1
			b. Hairs				
l1	214.7			h1	98.0		
		(present in 11.1 percent of the cases)					
l2	349.7			h2	96.5		
		(present in 38.9 percent of the cases)					
l3	505.1			h3	103.8		
		(present in 83.5 percent of the cases)					
l4	633.2			h4	118.0		
		(always present, but difficult to measure accurately)					

by the presence of two to five dorsocentrals per side per fly. Rarely an extra humeral is also present. The supernumerary dorsocentrals are found

in the dorsocentral line or an extension of it. Dorsocentrals were not commonly found anterior to the level of the transverse suture; those rare ones which did appear in this region were not included in the study. There was observed a continuous gradation from large hairs through small bristles to large bristles. Arbitrarily, setae are considered microchaetal if less than 200μ in length, and macrochaetal if longer.

Of the 102 *Pch* female half-thoraces drawn, 34 showed only *two dorsocentrals*. The mean locations and sizes of the bristles and hairs in these cases are given in table 2 and figure 3a. Comparison of this figure with figure 2, the wild-type pattern, reveals a great similarity between the two. However, every distance and length in the *Pch* flies is greater than the corresponding measurement in the wild-type. In order to determine whether differences in mean fly size play a role in this difference between the patterns, wild-type and *Pch* flies were raised under identical conditions. The average weight of 90 *Pch* females with only two dorsocentrals on one or both sides (usually the latter) was 2.18 mg. The average weight of 85 wild-type females was 1.94 mg. It will be noted that the heavier of the two kinds of flies is the one in which the chaetae are further apart and longer. In addition to the general size factor, there seems to be at least one specific distinction between the two patterns. The hairs anterior to B2 are not only absolutely but also relatively further apart than in the wild-type pattern, and one, the middle of the three, is longer. Otherwise, the patterns appear to be identical and will be treated as such in the following.

In a previous paper (NEEL 1940) it has been shown that in the case of a *Drosophila melanogaster* mutant, polychaetoid, which is morphologically very similar to *Pch*, there is a correlation between fly size and the number of dorsocentrals present per side. It seems probable that *Pch* behaves in a similar manner. This has a direct bearing upon the present studies. If *Pch* half-thoraces with two dorsocentrals are associated on the average with smaller flies than half-thoraces bearing three or four dorsocentrals, then too much emphasis cannot be placed upon a direct comparison of the measurements of these various patterns. While such comparison undoubtedly reveals in a general manner similarities and differences between various measurements, it cannot take into account the greater size of flies with higher bristle numbers.

The results of a study of 44 half-thoraces bearing *three dorsocentrals* are found in table 2 and figure 3b. In such cases B1 corresponds in size and position to the normal B1, and B2 is similar to the normal B2. B3 is found some 120μ anterior to the wild-type B2, near the normal locus of a large hair, and is in general shorter than B2; there is great variability in its length. A hair is found just ahead of the bristle in the p dc position, as

in the wild-type pattern, while between B2 and B3 there is usually (88.6 percent of the cases) found a second small hair, about the size of that normally directly in front of the a dc. There is, of course, a row of hairs anterior to B3, of which only the first is shown. Where a hair is an inconstant feature of a pattern, it is represented in this and subsequent figures by a broken line.

Occasionally (ca. 10 percent) a three-bristled condition has been observed in which bristles are present in the a and p dc positions, with the third located between these two. So far the number of cases is insufficient for analysis. However, a similar condition has been observed in *D. melanogaster pyd* and will be discussed below.

When *four dorsocentrals* are present per side (table 2 and figure 3c), B1 and B3 are in approximate correspondence with the normal positions and sizes of the p and a dc respectively. B4 is similar to B3 of the three-bristled cases, while B2 is about intermediate between the normal a and p dc positions. Both B4 and B2 are quite variable in size. The occurrence of hairs in the dorsocentral line differs among individuals; the frequency of appearance of one between B1 and B2, B2 and B3, and B3 and B4 is given in table 2.

Each of the bristles present in four-bristled flies has a characteristic size, resulting in a posterior-anterior size sequence of long-short-long-short. These mean differences between successive bristles along the dorsocentral line are fully significant, with the exception of that between B2 and B3, which is only 2.5 times the standard error of the difference ($29.9 \pm 11.9\mu$). It is apparent from both this and the three-bristled data that a supernumerary macrochaeta anterior to the locus of the a dc is on the average much smaller than that latter structure, while one between the positions of the a and p dc tends to be smaller than either.

Table 2 shows that as the number of bristles present on the thorax increases, the variability in the size of all those present rises. Thus, in all the patterns investigated B1 has been similar in mean size and position to the p dc, but for this particular bristle the standard deviation has risen from 18.4μ through 31.4μ to 71.2μ.

The wild-type pattern in Drosophila melanogaster

In table 3 and figure 4 the results of a study of 45 half-thoraces from wild-type (*Fla*) *Drosophila melanogaster* males are given. As would be expected from the size differences between the species, all dimensions of the *melanogaster* pattern are much less than the corresponding *funebris* measurements. Otherwise, the chief distinctions between the species are found in the relative sizes of the hairs which precede the a dc.

The coefficient of correlation between the distances L1 and (L2–L1) is

0.384 ± 0.127. In agreement with the conclusions reached earlier, it seems probable that size variations from fly to fly are a major contributing factor

TABLE 3

The mean positions and lengths of the dorsocentral bristles and hairs of wild-type Drosophila melanogaster males.

MEASUREMENT	M	σ_m	σ	MEASUREMENT	M	σ_m	σ
			a. Bristles				
L1	123.4	1.4	9.2	B1	336.2	3.7	24.7
L2	250.1	2.5	17.0	B2	250.1	2.5	16.7
			b. Hairs				
l1	172.5	2.2	14.9	h1	80.1	1.1	7.7
l2	301.1	3.4	21.6	h2	82.1	2.0	12.7
l3	335.0	3.9	22.4	h3	80.5	2.1	12.5
l4	373.1	4.0	23.2	h4	67.5	2.2	13.2

in this correlation. Size variations in this stock must therefore be relatively greater than in the *funebris* wild-type stock. The correlation between the lengths of B1 and B2 is 0.739 ± 0.067.

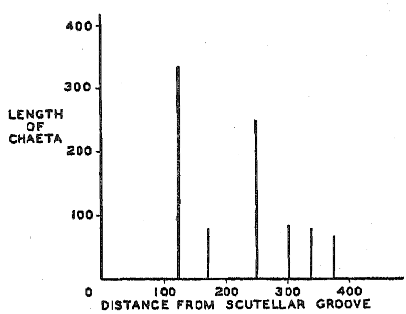


FIGURE 4.—Distribution and size of the dorsocentral bristles and hairs in wild-type *D. melanogaster* males.

Polychaetoid in Drosophila melanogaster

The weakly dominant mutant polychaetoid (*pyd*, $3-39\pm2$) in homozygous condition is characterized by the presence of supernumary bristles at many different loci. The dorsocentral region is particularly apt to be affected. The number of bristles present here may vary from two to, rarely, six per half thorax. Usually the extra bristles fall along the dorsocentral line, and cases where they deviate from the line have not been included in the study. In this mutant, as in *Pch* of *funebris*, hairs grade imperceptibly into bristles; the arbitrary lower limit for bristle size was set at 120μ.

A total of 108 *pyd* half-thoraces have been drawn, and in addition twice that many which due to some imperfection were unsuitable for drawing

have been looked over. Only the two-, the three-, and the four-bristled conditions have been studied. As pointed out earlier, there is a correlation between fly size and bristle number, and for this reason the two- and three-bristled conditions are on the average associated with a smaller half-thorax than the four-bristled. Comparison of the absolute measurements pertaining to the various patterns is only valid when the slight mean size differences between the groups of flies studied are kept in mind.

TABLE 4

The mean positions and lengths of the dorsocentral bristles and hairs of pyd Drosophila melanogaster males. In no case have the σ and σ_m of a measurement been calculated when the number of cases available is less than 20.

MEASUREMENT	M	σ_m	σ	MEASUREMENT	M	σ_m	σ
1. The two-bristled pattern							
			a. Bristles				
L1	100.3	1.2	6.6	B1	329.2	3.2	17.4
L2	210.8	2.8	15.2	B2	249.2	3.6	19.8
			b. Hairs				
l1	142.3	2.0	11.5	h1	71.6	2.0	11.5
l2	257.1	3.0	17.9	h2	82.9	3.1	18.0
l3	289.0	3.1	18.5	h3	75.7	2.7	16.2
l4	321.0	3.2	19.1	h4	66.4	1.7	9.5
2. The three-bristled pattern							
			Type 1. B2 close to B1				
			a. Bristles				
L1	96.4			B1	328.6		
L2	128.2			B2	257.0		
L3	224.5			B3	247.9		
			b. Hairs				
l1	181.2			h1	72.0		
l2	279.0			h2	83.4		
l3	310.1			h3	71.1		
l4	347.0			h4	66.3		
			Type 2. B2 not close to B1				
			a. Bristles				
L1	100.9	1.3	6.9	B1	339.2	3.7	20.4
L2	192.1	3.8	21.0	B2	263.5	6.5	35.8
L3	255.0	2.8	15.5	B3	203.5	8.3	45.6
			b. Hairs				
l1	133.5	2.3	10.1	h1	69.2	2.1	9.4
		(present in 67.8 percent of the cases)					
l2	210.4			h2	67.5		
		(present in 32.2 percent of the cases)					
l3	296.3	3.4	17.2	h3	74.3	1.5	7.4
l4	325.0	3.9	18.1	h4	69.5	1.3	6.2

TABLE 4—*Continued*

MEASUREMENT	M	σ_m	σ	MEASUREMENT	M	σ_m	σ
3. The four-bristled pattern. Measurements for the predominant (81.5%) type							
			a. Bristles				
L1	105.0	1.5	8.3	B1	375.6	5.0	28.0
L2	151.9	3.2	17.8	B2	261.3	12.4	68.1
L3	228.8	3.4	19.0	B3	289.7	7.4	40.3
L4	286.6	3.1	17.1	B4	208.7	7.7	42.2
			b. Hairs				
l1	189.7			h1	66.5		
		(present in 43.7 percent of the cases)					
l2	242.4			h2	64.5		
		(present in 21.9 percent of the cases)					
l3	338.6	3.4	18.2	h3	81.5	1.7	9.3
l4	369.6	4.2	22.0	h4	73.4	1.5	7.7

Thirty-one of the half-thoraces drawn bore only the *normal number of dorsocentrals* (table 4 and figure 5a). The only difference between *pyd* half thoraces with two dorsocentrals and the wild-type dorsocentral pattern is that all dimensions are less in the former. In agreement with this, microscopic inspection shows that *pyd* flies with two dorsocentrals on one or both sides are often appreciably smaller than the average wild-type fly studied.

A total of 184 half-thoraces with *three dorsocentrals* have been observed, and of these the 40 which showed no defects were drawn. All but three cases could be classified into one or the other of two alternative conditions. One group of flies (nine of the 40 drawn, 31.6 percent of the total 184 observed) is represented in table 4 and figure 5b. Comparison with the wild type or two-bristled *pyd* data reveals that in these type 1 cases, B1 shows a good correspondence to the *p dc* location, while B3 approximates the a dc position. B2 is found between the usual p and a dc locations, and is very similar in position to a hair in the wild-type pattern. This hair is now missing, but a "new" hair is found between B2 and B3.

The measurements of the remaining three-bristled cases (31 out of 40 drawn, 66.8 percent of the total 184 observed) are given in table 4 and figure 5c. In these type 2 cases B1 is found in the normal p dc position, B2 is at or somewhat posterior to the usual a dc location, and B3 appears some distance anterior to where a bristle normally occurs. In 67.8 percent of these cases there is a hair in the normal h1 position, between B1 and B2, but none between B2 and B3. In the other 32.2 percent the hair between B1 and B2 is missing, but one is now found between B2 and B3, and in these cases B2 is situated rather posteriorly. Anterior to B3 there is the usual row of hairs.

Although in both of these three-bristled patterns the size of B1 remains rather constant, that of the other two bristles fluctuates widely. On the average, when a macrochaeta comes between the usual positions of the p and a dc, it is not very different in size from the latter, while if it appears anterior to the position of the a dc it is considerably smaller.

A total of 119 half-thoraces with *four dorsocentrals* have been studied, of which 37 were suitable for drawing. Four different arrangements of the bristles were found: 1) In 81.5 percent (97 cases, 32 drawn) the pattern

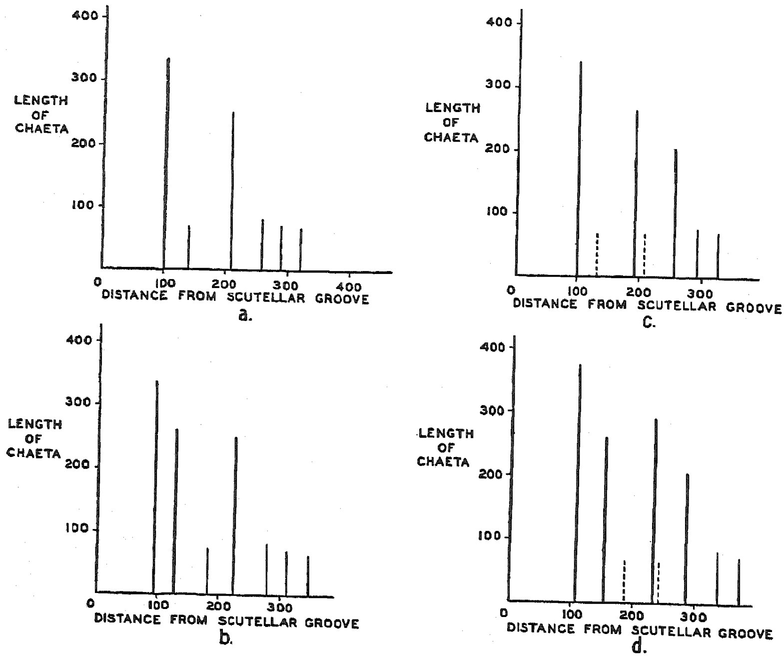


FIGURE 5.—The pattern of bristles and hairs along the dorsocentral line in *D. melanogaster pyd/pyd* males, when two, three, or four dorsocentrals are present per side. A hair which is an inconstant feature of a pattern is indicated by a dotted line.

of the four bristles was very similar (table 4 and figure 5d). Two of the four bristles are close to the normal p and a dc positions. Of the remaining, one is found between the a and p dc locations, either at or somewhat anterior to the position of the small hair which has been described. The other is found some distance anterior to the a dc location. A hair has never been observed between B1 and B2. In 43.7 percent of the cases there is one between B2 and B3, and in 21.9 percent, one between B3 and B4. The simultaneous appearance of hairs in both of these positions has never been noticed. In the figure, h2 is shown very close to B3. In no individual fly was such proximity observed; in those cases where h2 was in this posi-

tion, B3 was toward the posterior limit of its distribution. 2) In 10.9 percent (13 cases) the following was observed: bristles were present in approximately the normal a and p dc positions; a third bristle was present near where in the wild type h1 is found; the fourth was situated between the normal positions of h1 and the a dc (figure 6a). 3) A third pattern, seen in five cases, was characterized by bristles at the a and p dc locations, the appearance of a bristle anterior to the a dc position, and the appearance of a bristle very close to the p dc locus. In these cases the basal rings of B1 and B2 were often in contact (figure 6b). 4) In a fourth pattern (four cases) B1 was in the p dc position; B2 appeared somewhat posterior to the a dc location; B3, anterior to this locus; and B4 about 200μ from the p dc position (figure 6c). The three patterns shown in figure 6 are semi-diagrammatic.

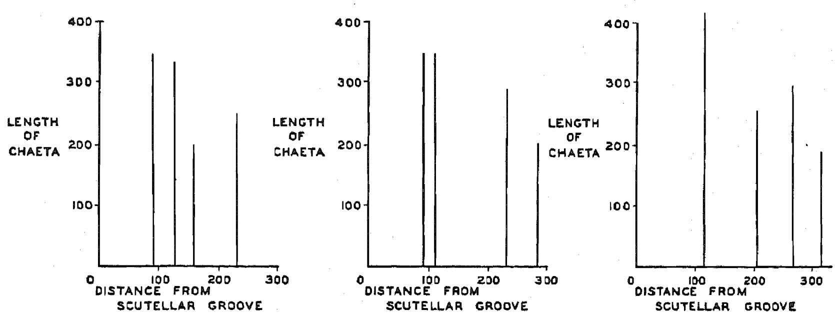


FIGURE 6.—Three additional patterns of the dorsocentrals when four are present per side in *pyd/pyd D. melanogaster* males. These together constitute 18.5 percent of all the four-bristled cases. Details in text.

In all of these four-bristled patterns it was found that bristles in the p or a dc locations tend to be larger than those located between these points, or anterior to the a dc location. Thus, the length differences between the bristles shown in figure 5d are all significant except that between B2 and B3, which is equal to two times the standard error of the difference (28.4$\pm$14.4μ). In this respect the pattern is very like the four-bristled *Pch funebris* arrangement, and serves to indicate that the size of a bristle is related to its location in the dorsocentral line.

In order to determine the relation between the number of dorsocentrals present on both sides of the fly and their total length, *pyd* male flies showing a wide range in bristle numbers were obtained by collecting from the same set of stock bottles over a 21 day period. Figure 7 shows that each bristle number tends to be characterized by a distinct range of total bristle lengths, although there is a definite overlap between the length distributions of consecutive bristle numbers. The observed overlap may

be due in part to errors in the evaluation of total bristle length. If the combined length of the dorsocentrals is an index of the amount of some hypothetical "bristle-forming" substance present, then it seems that in

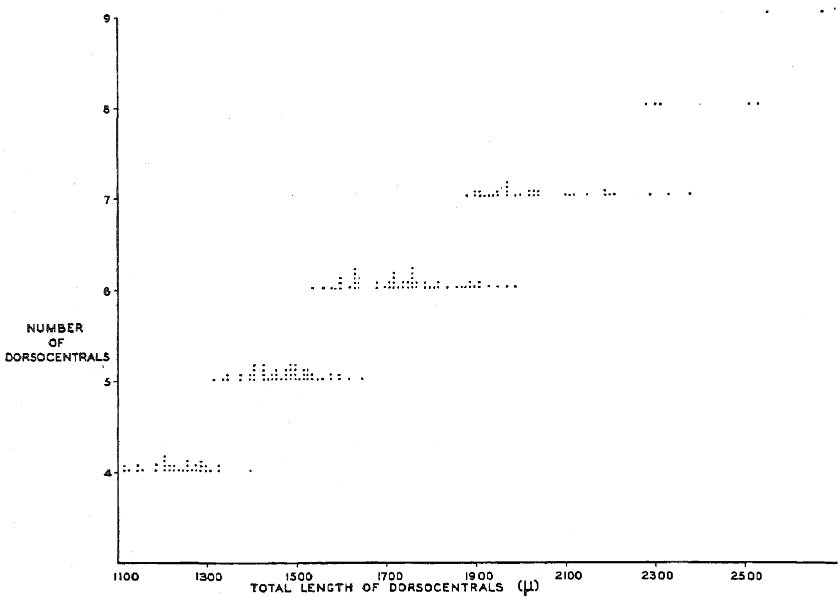


FIGURE 7.—The relation between the total number of dorsocentrals present in *pyd/pyd* males and their combined length. Each point on the figure represents a single fly.

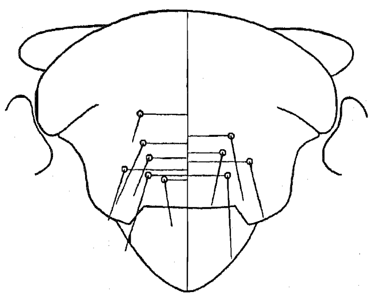
this mutant new bristles are called forth at regular intervals as the amount of bristle-forming substance increases.

Hairy wing in Drosophila melanogaster

Flies which are either homozygous or heterozygous for the dominant mutant Hairy wing (*Hw*, 1–0.0) possess extra hairs and bristles on the body and wing surfaces. DEMEREC and HOOVER (1939) have shown the *Hw* factor to be a short duplication for the salivary chromosome band 1 B 1.2. The expression of the factor was studied in *y Hw* dl–49 Theta/*y* sn^3 *bb* females. Since it is known that the Theta duplication contained in this stock by itself has a slight tendency to produce extra bristles, the appearance of supernumerary macrochaetae in these females cannot be attributed solely to the *Hw* factor. Unlike the patterns already considered, when supernumerary macrochaetae are present in the dorsocentral region they do not tend to fall along the dorsocentral line, but may show wide deviations from it. By using the midline and scutellar groove of these flies as the two axes of a rectangular system of coordinates, the position of each

bristle within the dorsocentral area could be expressed by two measured distances, namely, the distance from the scutellar groove and the distance from the midline. A sample case is shown in figure 8a.

In a wild-type Drosophila, and in *Pch* and *pyd*, there is a quadrilateral area between the dorsocentrals, on one hand, and the a sa, the p sa, and the a pa, on the other, in which macrochaetae are not present (see figure 1). In these *Hw* flies, bristles occur at almost any point in this region. Since the investigation concerns dorsocentrals and bristles which clearly arise in closer proximity to these than to any others, it becomes necessary to delimit a "dorsocentral area." This has been defined as shown in figure 8b. It is bounded medially by the midline; posteriorly, by a line parallel to the scutellar groove and 55μ anterior to this landmark; anteriorly, by



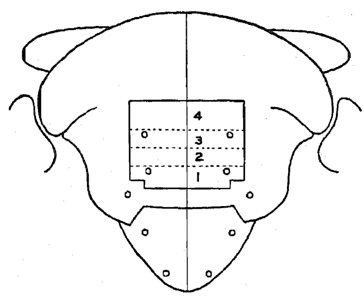


FIGURE 8a (left).—The dorsocentral bristles in a typical *y Hw* dl-49 Theta/ *y sn*^{3} *bb* female. The lines drawn on the camera lucida drawing of the thorax in order to determine the position of each bristle are shown; the most lateral bristle on either side is slightly outside the "dorsocentral area."

FIGURE 8b (right).—The "dorsocentral area" in *D. melanogaster*. The heavy line indicates the limits of this area, while the dotted lines show the levels into which the area was divided. Dimensions involved given in the text.

a second line parallel to the groove, 380μ in front of it; while the lateral boundary parallels the midline at a distance of 195μ from it except in the posterior 35μ of the area, where the lateral boundary is 150μ from the midline. The notch thus formed in the posterior-lateral corner of the area excludes from consideration bristles close to the p pa.

Figure 9 shows an anterior-medial view of a model of the area. The position and height of each bristle observed in this region on the 46 *Hw* half-thoraces studied is indicated in the figure by an insect pin. The standard of macrochaetae length used here was 100μ rather than the 120μ of *pyd* because of the smaller size of these *Hw* flies. From the model, a number of facts are evident. 1) There are two locations where there is a high frequency of occurrence of bristles. These are marked in the figure by arrows. These spots correspond to the positions of the normal p and a dc. Bristles found here tend to be larger than those found elsewhere. 2) Be-

tween the a and p dc locations, and medial to the p dc position, bristles also occur frequently and tend to be small. A few scattered macrochaetae, also small, were seen anterior to the a dc locus. 3) Bristles are more frequently found close to the midline in the posterior portion of the area than in the anterior.

The dorsocentral area was divided into four levels by lines drawn parallel to the scutellar groove at points 125, 195, and 265μ anterior to it (see figure 8b). In a wild-type fly, the p dc would be found in the first subdivision and the a dc in the third. For each level, the length of every bristle present was correlated with its distance from the midline. In each of the three posterior levels, a significant coefficient of correlation (r) was found. In level 1, r equals 0.836 ± 0.037; in level 2, 0.451 ± 0.123, and in

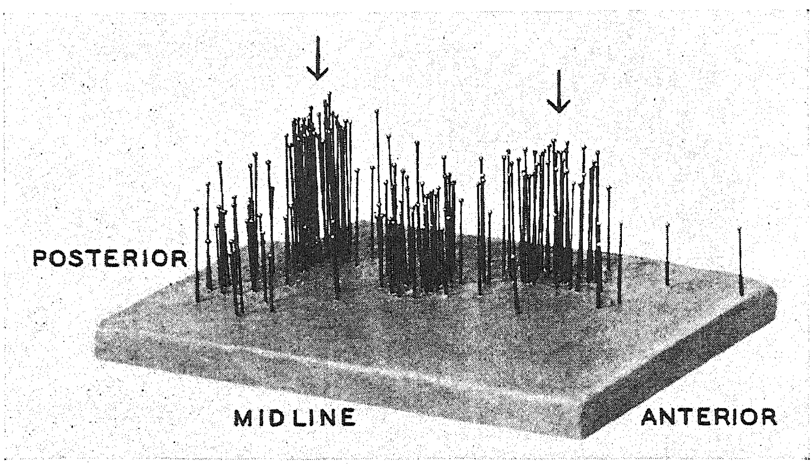


FIGURE 9.—The distribution and size of the bristles observed in the "dorsocentral area" of *y Hw* dl-49 Theta/ *y sn³ bb* females.

level 3, 0.711 ± 0.076. The number of bristles present in the fourth level is too small for a calculation of r. Nevertheless, it seems clear that throughout the dorsocentral area bristle length tends to be an inverse function of nearness to the midline.

If one subdivides each level into a more median (0–100μ) and a more lateral (100–195μ) zone one finds that each of the four latter is characterized by a significantly different mean bristle length as follows (proceeding anteriorly): $276.7 \pm 3.9\mu$, $179.5 \pm 6.3\mu$, $220.7 \pm 5.3\mu$, and 202.8μ. In the last level no standard error of the mean has been calculated because of the small number of cases (17). The long-short-long-short sequence of mean bristle sizes in the lateral halves of the four levels is quite comparable to that observed in four-bristled *pyd* and *Pch* flies. The occurrence of extra bristles in the median zones is rare except in the most posterior level.

Those found here have a mean size similar to that of the bristles in the lateral zone of level 2.

The scutellar bristle pattern in pyd Drosophila melanogaster

The question of whether extra bristles in another area of the thorax are subject to the well-defined position and size tendencies shown by those in the dorsocentral region was next investigated. The scutellum in *pyd* males was studied, since it is available for an accurate analysis and is often marked by extra bristles.

In *pyd* flies there may be none, one, or two extra scutellars per side. When there are no supernumerary bristles the pattern is in no respect different from the wild type (figure 10a, based on 21 cases). If only one extra macrochaeta is present, it is characteristically found close to either the a sc or p sc position, and only occasionally intermediate to the two loci.

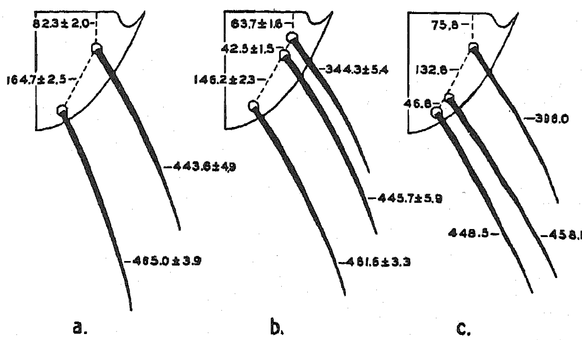


FIGURE 10.—The pattern of the scutellar bristles in *pyd*/*pyd* males which have either two or three of these per side per fly.

Accordingly, on the basis of the position of the extra bristle—whether it is close to the a sc or p sc location—two types of *pyd* flies with one extra scutellar can be distinguished. When the supernumerary macrochaeta is close to the a sc locus, the average relationship of the three bristles now present is indicated in figure 10b (28 cases). As regards position, neither of the two anterior bristles corresponds to the a sc, since one occurs some 20μ anterior to the normal location and the other an equal distance posterior. With respect to size, the posterior of the two is very like the normal a sc, while the more anterior is characteristically smaller. This is a regular difference, comparable to the size-place relationships seen in the dorsocentral region. The third bristle resembles the p sc. Figure 10c shows the average of 13 cases where the supernumerary scutellar is in the p sc region (no σ_m is given because of the small number of cases). The anterior of the three bristles now present resembles the a sc; the most posterior is similar

to the p sc; while the middle, although similar in size to the p sc, is some 40μ anterior to the wild-type position of this macrochaeta. These two extra-bristled positions are not the average of wide deviations about a mean, but are rather constantly adhered to in any given case. Only a few flies with four scutellar bristles on one side have been found; in these instances one extra bristle was in the a sc region and the other anterior to the p sc location.

The innervation of supernumerary bristles

STERN (1938), using the methylene blue technique of intravitam staining, found that each hair and bristle of Drosophila is innervated by a separate bipolar cell situated below the hypodermis. From each nerve cell one short fiber is sent to the base of the seta, while the second long fiber joins with similar long fibers from other cells into nerves which lead to the central nervous system. A cell innervating a bristle is larger than one innervating a hair. It was noted that the nerve cell supplying the a dc sends its axon directly anteriorly to join a rather small nerve trunk, while the axon of that innervating the p dc runs posteriorly and laterally to join a large trunk.

In order to determine what the relation is between bristle and nerve patterns, the source of innervation of the supernumerary dorsocentrals of *pyd* flies was studied by means of the methylene blue technique. It was found that with very few exceptions, nerve cells supplying bristles at the p dc position or between the normal a and p dc positions send their axons to join the large posterior trunk to which the nerve cell of the p dc normally contributes. The axons of nerve cells connecting with bristles at or anterior to the a dc position joined the same trunk with which the nerve cell of the a dc normally establishes connections. This is true regardless of the exact pattern of the bristles, that is, in type 2 three-bristled *pyd* (figure 5c), when B2 was found as far forward as the normal a dc, it was innervated from the front, but otherwise, from the rear. A specific bristle pattern is not correlated with a particular type of bristle innervation.

The development of the bristles

It has been seen that whenever more than the normal number of dorsocentrals are present, and particularly in *Pch* and *pyd* when four are found on one side, characteristically the largest are found in or close to the normal p and a dc positions. Likewise, large differences are observed in the size of the various bristles composing the normal pattern. A relation between the position on the thorax of a bristle and its size is evident. Two possibilities concerning the nature of this size restriction present themselves. 1) All the bristles may appear at the same stage in development but

grow at rates which differ from region to region of the thorax, or 2) those bristles which are to be the largest may begin to differentiate first and retain their initial advantage throughout development. According to this latter hypothesis, the size differences between hairs and bristles might be due to a later onset of development in the former.

ROBERTSON (1936), working on *Drosophila melanogaster*, reported that "the mechanism of formation of hairs and bristles appears to be identical, both being formed at the same time" (p. 365). According to this author, at 25°C the trichogenic cells which enter into the formation of bristles may first be seen about the twenty-seventh hour of pupal life; the bristles appear shortly after the thirtieth hour as colorless extensions of the trichogen. The abdominal bristles appear a little later than those on the anterior part of the body.

MR. HARRISON STALKER of this laboratory has kindly placed at my disposal a series of slides of early wild-type Drosophila pupae. ROBERTSON'S observations with respect to the time of appearance of the macrochaetae were confirmed. The bristle-forming trichogens could be located at an earlier hour than the hair-forming, but the smaller size of the latter militates against their being seen. Likewise, although the initial outgrowth of both hair and bristle-forming cells is colorless, the smaller size of the former makes it much more difficult to identify. For this reason it has not been possible to determine with certainty whether hairs and bristles appear simultaneously; there may be a slight difference, with bristles appearing first. No difference in the order of appearance of the various thoracic bristles has been established, although inequalities in adult bristle length are foreshadowed at an early stage in pupal development. If the hairs and bristles do not appear simultaneously, then the time interval between the formation of each must be small.

The effect of these mutants upon the bristle pattern as a whole

PLUNKETT (1926) in an extended analysis of the Dichaete mutant showed that the bristle-removing effect of this factor was strongest in the vicinity of the ps bristle. As distance over the thorax from this locus increased, the effect of the gene tended to decrease, although in a somewhat irregular manner. It was further found that three other bristle-removing genes showed a region or regions of maximum effect. ROKIZKY (1930) reports similar findings.

Hw, *Pch* and *pyd* have been investigated with regard to an area of maximum effect. Since ROKIZKY has already reported on *Hw*, no reanalysis of this mutant was undertaken. His observation, that extra bristles occur most frequently in the dorsocentral region, has been confirmed. The *Pch*

mutant is extremely localized in its action in the stock employed. Extra bristles, common in the dorsocentral region, have been observed at only one other locus, the humeral, and there but seldom. However, N. W. and H. A. TIMOFEEFF-RESSOVSKY (1934) and N. W. TIMOFEEFF-RESSOVSKY (1935) have shown that the mutant under suitable conditions may remove bristles, and have found that in one of their stocks the greatest bristle-adding effect of the gene is on the anterior dorsum of the thorax whereas in another it is on the posterior portion of this region.

The *pyd* factor is quite general in its effects. In figure 11 the average number of bristles present at or near each of 20 loci, on 100 half-thoraces, has been indicated. At only four of these loci have extra bristles never been found. The maximum effect of the gene is on the dorsocentrals, with

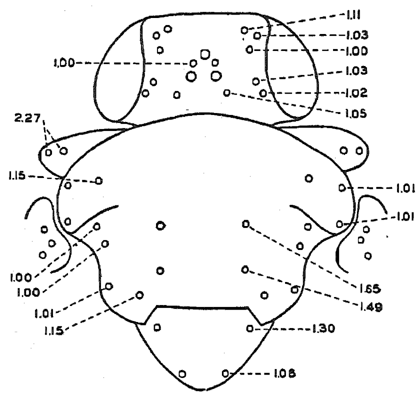


FIGURE 11.—The frequency of occurrence of bristles in *pyd/pyd* males at or near each of twenty loci.

scutellar, presutural, and humeral bristles also frequently affected. (Inasmuch as it was often found impractical to distinguish a supernumerary macrochaeta as u h or l h, these two loci have been treated as one.)

In an attempt to explain this characteristic 'field effect' in the case of Dichaete, PLUNKETT (1926) has postulated that during early embryonic development a percursor of a catalyst of a bristle-destroying reaction diffuses out from two bilaterally symmetrical centers in the egg. These centers correspond to the region of greatest effect of Dichaete in the adult. In explanation of the pattern of missing bristles seen in scute flies, STURTEVANT and SCHULTZ (1931) and GOLDSCHMIDT (1931) have likewise advanced hypotheses calling for diffusion from one or several centers.

However, MULLER (1932), from data accumulated in the Austin laboratory on gynandromorphs involving various scute alleles, has concluded that "the development of the bristles, insofar as it is under the influence of the scute gene, is not governed by one or a few centers, but is in its

major features autonomous at the site of each bristle" (p. 250). CHILD (1935) found in scute flies a complete absence of association between the bristles of the same side, as measured by the association coefficient. He therefore concluded that the concept of a pattern in scute was based upon nothing more than a difference in mean numbers, in a group of flies, of different bristles, and did not correspond to any coordinating developmental process in the individual fly (such as a diffusion phenomenon). IVES (1939) has reported similar findings. However, in Dichaete flies high association coefficients between adjacent bristles were reported (PLUNKETT 1926).

The 15 possible association coefficients between the six bristle loci (humeral, ps, a dc, p dc, p pa, and a sc) most frequently affected in *pyd* male flies have been calculated. The u h and l h have been treated as one in the calculation of the coefficients. For none of the 15 possible combinations of these loci was there found a significant association coefficient. As in CHILD's studies, so here it may be concluded that each bristle locus behaves independently of the others.

TABLE 5

Right-left correlation coefficients for all combinations of the humerals, ps, a dc, p dc, p pa, and a sc in pyd male flies. The 100 flies used in the derivation of these correlations were raised under slightly adverse cultural conditions.

		LEFT SIDE					
		humerals	ps	a dc	p dc	p pa	a sc
RIGHT SIDE	humerals	0.352 ±0.088	0.061 ±0.099	0.001 ±0.100	−0.042 ±0.100	0.126 ±0.098	−0.173 ±0.097
	ps	−0.100 ±0.099	0.156 ±0.098	0.065 ±0.099	−0.040 ±0.100	−0.038 ±0.100	0.155 ±.0098
	a dc	0.039 ±0.100	0.093 ±0.099	0.173 ±0.097	0.124 ±0.098	0.142 ±0.098	0.096 ±0.099
	p dc	0.020 ±0.100	−0.104 ±0.099	0.064 ±0.099	0.376 ±0.086	−0.111 ±0.099	0.013 ±0.100
	p pa	−0.038 ±0.100	0.187 ±0.096	0.110 ±0.099	0.168 ±0.098	0.058 ±0.099	0.024 ±0.100
	a sc	−0.202 ±0.096	0.034 ±0.100	0.013 ±0.100	0.002 ±0.100	0.035 ±0.100	0.289 ±0.092

Nevertheless, there are indications that in *pyd* flies the appearance of extra bristles at the various loci affected is not governed by chance alone. One hundred male flies were taken from a lot subjected during develop-

ment to overcrowding. Six bristle loci were studied: humeral, ps, a dc, p dc, p pa, and a sc. For every locus, the coefficient of correlation for the presence of extra bristles at it and at each of the six loci on the opposite side was calculated. Thus, a total of 36 coefficients was derived (table 5). In three instances significant left-right correlations were found. In each of the three cases, the correlation was between homologous bristles on the two sides, rather than between non-homologues. The implications of this are clear. The two half-thoraces which together constitute the right and left halves of a single fly have developed under as nearly identical conditions as can be obtained. The environmental conditions which tend to produce an extra bristle at one locus tend also to produce an extra bristle at the corresponding locus on the other side.

DISCUSSION

The data presented in the last section show that in *pyd* flies the macrochaetae found at or near a given bristle locus vary independently of those found at nearby loci. A similar independence of the bristle loci has been noted in *Pch* and *Hw*. This finding justifies a study of a few bristles or groups of bristles as a means of getting at the manner of action of these mutants.

The position of the bristles

In a consideration of the positions of the bristles composing the various patterns seen for each mutant, two possibilities at once present themselves. 1) The various patterns of, for example, *pyd* flies may be related to one another in the sense that a given bristle or hair of one pattern corresponds to (is homologous with) a bristle or hair of another pattern. 2) It is also possible that the different patterns are in no wise related to one another, but that each represents a totally different expression of the activity of the gene.

In every mutant pattern studied, there is a bristle which shows an excellent correspondence to the p dc. For instance, B1 of the three- and four-bristled *pyd* patterns closely resembles B1 of two-bristled *pyd* (figure 5), and this latter, we have seen, corresponds to the normal p dc (figure 4). The same similarity of B1 to the p dc is true of all *Pch* patterns (figure 3).

Likewise, in every mutant pattern a bristle resembling the a dc is found, although this resemblance is not as close as that noted for the p dc. Thus the position of B2 in the two-bristled *Pch* dorsocentral pattern may be compared with that of B2 in the three- and B3 in the four-bristled patterns (figure 3). In every case there is an approximate correspondence to each other and to the normal a dc. However, the exact mean distances of the three bristles from the scutellar groove are the following: in two-bristled, $437.6 \pm 2.9\mu$; in three-bristled, $415.7 \pm 4.2\mu$; and in four-bristled,

$470.8 \pm 5.6\mu$. These significantly different positions cannot be explained in terms of mean size differences; if this were the case B2 of the three-bristled pattern should be intermediate to the other two in its distance from the scutellar groove. Therefore, if these three bristles are homologous, some factor in development other than size has brought about significant shifts from pattern to pattern in the mean positions of the chaetae-forming cells involved.

Another example of an approximate correspondence of a bristle of a mutant pattern to the normal a dc is provided by B2 of the type 2 three-bristled *pyd* condition (figure 5c). This bristle differs from the normal a dc chiefly in that its mean location is $18.4 \pm 4.7\mu$ posterior to the normal position. In individual cases the location of the bristle is rather variable. On the assumption, in some instances, of a posterior shift in the chaeta-forming cell involved, it could be identified with B2 of the normal pattern. Such an assumption, although appealing, has at present no supporting evidence.

Comparisons of several of the mutant patterns with wild type reveals a striking coincidence between the position of a hair in the wild type and the position of a bristle in a mutant pattern. Thus, B3 of the three-bristled *Pch* pattern (figure 3b) shows a correspondence to the large hair normally found anterior to B2 of wild type and two-bristled *Pch* flies (figures 2 and 3a). As another example, B2 of the type 1 three-bristled *pyd* dorsocentral pattern (figure 5b) resembles in position h1 of the wild-type pattern, and when this bristle is present, h1 is missing. B2 of the predominant four-bristled pattern (figure 5d) also is located where h1 normally is found. The agreement in position is so striking that it seems to call for some kind of causal interrelationship; it seems quite probable that the anlage which would normally form a hair may on occasion hypertrophy and form a bristle.

There are, on the other hand, instances of bristle occurrence where there is no correspondence to a hair or bristle of the normal pattern. Thus B2 of four-bristled *Pch* (figure 3c) does not closely resemble any feature of the normal pattern.

H. A. and N. W. Timofeeff-Ressovsky (1934) in their study of *Pch* found that the first extra dorsocentrals to appear usually came between the a and p dc positions. When many extra bristles were present on the anterior dorsum of the thorax, no bristle was found in the p dc locus. They suggest, without making a detailed study, that *Pch* may cause hypertrophy of hair anlagen.

Since in levels 1 and 3 of the dorsocentral area of *Hw* flies there are found macrochaetae corresponding to the p and a dc, respectively, it seems that the effect of *Hw* is to add bristles to the normal pattern rather than to

substitute an entirely new pattern. It is noteworthy that in this mutant the relations of an extra bristle in the dorsocentral region to the surrounding hairs are similar to the relations of a hair occurring in the same region to the other hairs. The data do not permit a decision as to whether hair anlagen hypertrophy into bristles, or whether bristles may occur at any point on the thorax in complete independence of the hair pattern. The latter alternative would require that with the determination of the occurrence of a bristle at any one point there follows a rearrangement of the hair anlagen about that place, so that the distances between hair and bristle are still of the order characteristic of those between two hairs.

In the final analysis, any decision between the two possible explanations of the positions of the bristles suggested above is not feasible with the present data. That the various patterns observed are derived from normal is suggested by the fact that there is always present a bristle closely corresponding to the p dc, and also one approximating the a dc. Moreover, the repeated coincidence in the position of a hair in the wild-type pattern and a bristle in a mutant pattern suggests that a hypertrophy of a microchaetal anlagen into a macrochaeta is one and perhaps the usual mode of action of these mutants, although hair anlagen hypertrophy clearly cannot explain all occurrences of extra bristles.

The size of the bristles

We have seen that in these mutant patterns the largest bristles occurring in the dorsocentral area are usually found in or close to the a and p dc locations. The only pattern where this localization is not apparent is type 1 three-bristled *pyd*. If the size of the bristles present in the various regions of the dorsocentral area be regarded as an adequate expression of "bristle-forming potencies," then a regular gradient in these potencies is indicated. However, from the standard deviation values given in the tables, it is clear that the individual bristles of a region vary considerably about the mean; the manifestation of the gradient is variable from fly to fly.

The fact that there is a gradient on the thorax in bristle-forming ability, which is only revealed when supernumerary bristles appear, presents an interesting analogy to a case described by D'ARCY THOMPSON (1917) and HUXLEY (1932). In the one-horned rhinoceros the single median horn curves backward due to the more rapid growth of the anterior surface. In the two-horned species the second horn, located posterior to the first, is smaller and less curved. This implies "that the growth-gradient made visible in the form of the anterior horn is continued across to the second horn-area, causing the growth intensity to diminish, and therefore resulting in a smaller horn . . . we must assume that even in one-horned rhinoceroses,

the growth-gradient is continuous along the head, but can only reveal itself in species where a second specific horn-area is present" (HUXLEY 1932, p. 152).

The nature of the bristle pattern

From the present data some very tentative inferences may be drawn as to the nature of the normal and mutant bristle patterns studied. The unit of the pattern is the individual bristle. This is shown by the manner in which the loci vary independently of one another. In the dorsocentral region each of the bristles present in wild-type flies arises in the center of a field of bristle-forming potencies. Whether this is true of all other loci is not clear; certainly there is evidence for localization of bristle-forming abilities in the scutellum. Since there are wide variations in the size of the bristles appearing at any one spot on the thorax, the gradient cannot be regarded as extremely rigid in its specifications. This field would seem from the embryological evidence to be primarily due to some spatial difference in the distribution of bristle-forming materials, rather than to a temporal difference in the time of appearance of various chaetae. It is not bound up with the manner of innervation of the bristles.

The mechanism which normally brings about the appearance of a bristle in the center of the field and only there is unknown. It is obvious, however, that in certain mutant conditions this mechanism is disrupted, and bristles may appear at almost any point in the field. These extra bristles may be the result of hypertrophy of microchaetae-forming cells, or may be structures not foreshadowed in the normal pattern. In some instances where it seems that a bristle of a mutant pattern corresponds to a macrochaeta of the normal pattern, at the same time it is probable that the position of the bristle has been somewhat shifted from the normal.

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SUMMARY

1. Five genotypes have been studied with respect to the size and position of the bristles: wild type and *Pch* of *Drosophila funebris;* and wild type, *pyd*, and *y Hw* dl–49 Theta/*y* sn^3 *bb* of *D. melanogaster*. Each of the mutant conditions is characterized by the presence of extra bristles.

2. For each mutant genotype a number of clearly defined and alternative patterns of the bristles were observed at the bristle loci investigated. These patterns are described and compared with the wild type.

3. When supernumerary macrochaetae are found in the dorsocentral region, two of the three or more bristles now present tend to correspond in size and position to the wild-type p and a dc. Frequently the position of an extra bristle coincides with that of a hair in the wild-type pattern. It is suggested that the factors for supernumerary bristles may act through the medium of hair anlagen hypertrophy. However, this is clearly not the only way these mutants work, for extra bristles regularly occur in normally hairless areas.

4. In the presence of more than the normal number of dorsocentrals, the largest characteristically are found in or close to the normal a and p dc positions. As distance from these points increases in any direction, there is a tendency for bristle size to decrease.

5. Supernumerary bristles on the scutellum of *pyd* flies are found close to the normal a and p sc positions; there is a relation between the size and the position of a bristle.

6. There is no association of a particular bristle pattern with a particular manner of innervation of the bristles.

7. Either all the macro- and microchaetae begin to differentiate simultaneously during early pupal life, or such differences in the order of appearance as do exist are small.

8. The absence of significant association coefficients between the chief bristles affected by the *pyd* factor shows that these vary independently. The existence of significant left-right correlations between homologous bristle loci on the two sides, while non-homologues show no such relation, suggests that the bristle pattern on one side of the fly is the result of a definite developmental condition frequently duplicated on the opposite side.

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GENETICAL STUDIES IN CULTIVATED RASPBERRIES. II. SELECTIVE FERTILIZATION

D. LEWIS

John Innes Horticultural Institution, Merton, England

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INTRODUCTION

DISTURBED gene segregations have been frequently observed in plant genetics, but in comparatively few cases has the cause of the disturbance been elucidated. This lack of information is probably due to the general practice of selecting a strain which segregates normally for any character under investigation and neglecting strains which give abnormal ratios, thus simplifying the interpretation of the results. In the cultivated raspberry the genes T and G had frequently given aberrant ratios (CRANE and LAWRENCE 1931, LEWIS 1939) but it was not until a series of T:G linkage crosses had been analyzed that the probable cause of the aberrant ratios was evident. It was shown that the segregation of the genes T and G was heterogeneous in F_2 and backcross families in which the male was heterozygous, but that female heterozygous backcross families gave normal ratios. In this paper the whole of the results are fitted into a general scheme and the cause of disturbed segregations given.

ABERRANT RATIOS IN LINKAGE FAMILIES

The results of some of the more critical families are given in table 1. Significant deviations from the expected 1:1 ratio for both T:t and G:g are found in the family 19/36, in which the male parent is heterozygous for both these genes. Gene ratios in the reciprocal cross closely approximate to the expected 1:1 ratio. In the selfed family the T:t segregation deviates significantly from the expected values and the G:g segregation deviates, although not significantly, in the same direction as the G:g segregation in the backcross, that is, there is a deficiency of recessives in both families. The close fit obtained between the observed figures in family 22/36 and the expected values which have been calculated from family 19/36 show that the deficiency of T and g types is consistent in the male heterozygous backcross and in the selfed heterozygote families.

In these families T and G are in the repulsed phase, therefore a deficiency of plants carrying T is accompanied by a deficiency of plants carrying the recessive allele of the other gene.

These abnormal ratios and the heterogeneity described in a previous publication can be explained on the hypothesis that there is a gene w linked with t and g which has a differential effect on the male gametophyte, but

has no influence on the female gametophyte. The growth of pollen tubes carrying the gene *w* is retarded or completely arrested at some stage between pollen germination and fertilization, so that only a small proportion or none of the *w* pollen reaches the egg cells. The only manifestation of the gene is the disturbance of the $T{:}t$ and $G{:}g$ ratios. As some plants may be homozygous *W W* or have the genes in linkage or repulsion, heterogeneity

TABLE I

FAMILY	CROSS	RATIOS		χ^2	p
		T	*t*		
19/36	2-3/32 (*t g*/*t g*)	44	103	23.6801	<.01
		73.5	73.5		
	×				
		G	*g*		
	2-8-6/32 (*T g*/*t G*)	107	40	30.5374	<.01
		73.5	73.5		
		T	*t*		
21/36	2-8-6/32 (*T g*/*t G*)	79	70	0.5436	.50–.30
		74.5	74.5		
	×				
		G	*g*		
	2-3/32 (*t g*/*t g*)	70	79	0.5436	.50–.30
		74.5	74.5		
		T	*t*		
22/36	2-8-6/32 (*T g*/*t G*)	60	35	7.1052	<.01
	selfed	71.25	23.75		
		*61.7	33.3	0.1350	.80–.70
		G	*g*		
		78	17	2.5573	.20–.10
		71.25	23.75		
		*81.9	13.1	1.4177	.30–.20

* Calculated from the observed figures in family 19/36.

as well as abnormal ratios would be expected when the genes are segregating on the male side. This is in complete agreement with the results.

On the above assumption, the crossover values between *T*, *G* and *W* can be calculated if the small proportion of *w* pollen which is effective in fertilization is known. Unfortunately, the available data supply no information on this fraction, but it is of interest to determine the relationship between it and the crossover value

If x = fraction of *w* pollen which is effective in fertilization

q = crossover value between *G* and *W*

q′ = crossover value between *G* and *W* when x = zero, then the expected types when *G* and *W* are coupled are:

$$\begin{array}{lll} 1-q' & \begin{cases} G\,W & (1-q) \\ G\,w & qx \end{cases} \\ q' & \begin{cases} g\,W & q \\ g\,w & x(1-q) \end{cases} \end{array}$$

therefore

$$q' = q + x - qx$$
$$q' = q(1 - x) + x$$
$$q = \frac{q' - x}{1 - x}.$$

The value of x must lie between zero and q′, since q cannot have a negative value. If x = q′, *g* and *w* are at the same locus. The assumption that x = zero has been made in the calculation of the crossover values, in other words that pollen carrying *w* is completely inhibited.

THE CALCULATION OF CROSSOVER VALUES

The whole of the relevant data have been analyzed, so that a fairly accurate estimate of the crossover values might be determined. The data of *T*:*t* segregation are given in table 2. The F_2 and male heterozygous backcross families have been grouped into three classes: 1) Families with a

TABLE 2

Selfed or intercrossed heterozygotes.

(*T W*—coupled)

	Parents	*T*	*t*	a^2/n	Heterogeneity χ^2	D.F.	p
7/36	L.G.×P.R.	148	29	4.7514			
10/37	1-5-10/33×1-29/35	151	33	5.9186			
13/37	12-18/35×12-9/35	402	80	13.2780	9.5000	4	.05—.02
14/37	12-18/35 selfed	187	32	4.6757			
4/31	1-24/27 selfed	109	7	0.4224			
	Total	997	181				
	(*T w*—repulsed)						
9/37	1-5-10/33 selfed	231	102	31.2432			
11/37	12-9/35×1-5-10/33	186	94	31.5571	1.4848	2	.50—.30
22/36	2-8-6/32 selfed	60	35	12.8947			
	Total	477	231				
	MALE HETEROZYGOUS BACKCROSSES						
	(*T W*—coupled)						
28/36	2-8-5/32×2-7/32(*g g*)	56	36	14.0869			
8/36	15-34-17/31×L.G. (*G g*)	41	19	6.0166	1.8822	2	.50—.30
14/36	M-6/29×7-15-50/31(*G g*)	61	26	7.7701			
	Total	158	81				
	(*T w*—repulsed)						
19/36	2-3/32×2-8-6/32 (*G g*)	44	103				

TABLE 2—*Continued*

	FEMALE HETEROZYGOUS BACKCROSSES					
1/29	L.G.×9-6-7/27(*g g*)	85	95			
2/29	L.G.×9-5-5/27(*G g*)	51	36			
4/29	1-47/25×2-59/25(*G G*)	54	60	3.8804	3	.30—.20
21/36	2-8-6/32×2-3/32(*g g*)	79	70			
	Total	269	261			
	SELFED OR INTERCROSSED HETEROZYGOTES AND BACKCROSSES (normal ratios)					
		T	t			
9/36	B.H.×L.G.(*G g*)	23	9			
10/36	B.H.×P.R.(*G g*)	44	9			
8/37	B.H.×1-5-10/33(*G g*)	109	41			
1/32, 2/33	B.H. (*G G*) selfed	290	90			
15/36	2-1/33×B.H. (*G G*)	21	5			
26/36	1-2-11/32×B.H.(*G G*)	70	20			
12/36	1-5-10/32×B.H.(*G G*)	155	61			
13/35	B.H.×1-25/32(*G G*)	57	21			
4/36	2-1/33(*G G*) selfed	85	27			
11/36	B.H.×1-2-11/32(*G G*)	56	23			
25/36	1-2-11/32(*G G*) selfed	67	32			
20/35	14-33-45/31(*G G*) selfed	21	10			
3/31	1-11/27(*G G*) selfed	36	7			
8/31	1-78/28×S.(*G G*)	194	54			
9/31	2-3-43/28(*G G*) selfed	122	35			
3/33	7-60/31(*G G*) selfed	30	8			
3/34	8-19-41/31×8-19-4/31(*G G*)	27	4			
11/37	1-5-10/33×12-18/35(*G g*)	161	64			
6/29	9-5-3/27(*G g*) selfed	37	8			
8/29	9-5-8/27(*G g*) selfed	65	19			
10/29	9-3-11/27(*G g*) selfed	23	9			
5/31	9-5-4/27(*G g*) selfed	259	74			

L.G.=var. Lloyd George; P.R.=var. Pyne's Royal; B.H.=var. Burnett Holme; S=var. Superlative.

significant deficiency of the recessive class (coupling). 2) Families with a significant deficiency of the dominant class (repulsion). 3) Families with normal ratios. It is possible to make errors of grouping with this method, particularly if the families are small. However, the chances of error from this source are reduced, since the families are large. The exact treatment of such data has not been worked out and in any case would be extremely laborious.

The best method of grouping, that is, on the male parents, is unsuitable for the T:t data because of the large number of different male parents involved. It would produce a large number of small groups, thus defeating the object of grouping. Fortunately it has been practicable to use this method for the G:g data because of the small number of different male parents. It is reassuring that in the T:t data, wherever a male parent

has been used more than once, it always appears in the same group, for example, 12-9/35 appears twice in group 1 but does not appear in group 2. Conversely 1-5-10/35 appears twice in group 2, but does not appear in group 1.

On the assumption that pollen carrying *w* is completely inhibited at some stage before fertilization, the expected frequencies when p is the crossover value between *T* and *W* are given in table 3.

TABLE 3

	EXPECTED		OBSERVED	
	T	*t*	*T*	*t*
Backcross coupling	1−p	p	158	81
Backcross repulsion	p	1−p	44	103
F_2 coupling	2−p	p	997	181
F_2 repulsion	1+p	1−p	477	231

The logarithm likelihood expression for the data in table 3 is:

$$L = 306 \log p + 492 \log (1-p) + 997 \log (2-p) + 477 \log (1+p).$$

On maximizing by differentiation and equating to zero, the value of p obtained is $.3201 \pm .0151$ to four decimal places. The data are homogeneous, χ^2 being 1.6515 for three degrees of freedom, p = .70 − .50. The method of calculation of the standard error and heterogeneity χ^2 is due to MATHER (1938).

A similar procedure has been adopted for the *G*:*g* data, which are given in table 4, except that the grouping has been based on the male parent.

TABLE 4

Selfed or intercrossed heterozygotes.

		G	*g*	a^2/n	Hetero-geneity χ^2	D.F.	p
9/27	L.G. selfed	77	14	2.1538			
12/35	P.R.×L.G.	18	2	0.2000	0.3747	2	.90−.80
8/36	15-34-17/31×L.g.	50	9	1.3728			
	Total	**145**	**25**				
5/36	L.G.×15-34-17/31	79	11	1.3444			
12/36	3-27/29×15-34-17/31	54	5	0.4237	1.3246	2	.70−.50
13/36	3-64/29×15-34-17/31	55	4	0.2711			
	Total	**188**	**20**				
3/29	L.G.×P.R.	76	9	0.9529			
7/36	L.G.×P.R.	82	8	0.7111	1.4181	2	.50−.30
9/29	P.R. selfed	43	2	0.0888			
	Total	**201**	**19**				
8/29	9-5-8/27 selfed	68	16	3.0476			
6/29	9-5-3/27 selfed	31	14	4.3555			
10/29	9-3-11/27 selfed	27	5	0.7812	6.6779	4	.20−.10
5/31	9-5-4/27 selfed	264	69	14.2972			
2/29	L.G.×9-5-5/27	61	26	7.7701			
	Total	**451**	**130**				

TABLE 4—*Continued*

22/36	2-8-6/32 selfed	78	17	3.0421			
14/36	M-6/29×7-15-50/31	60	27	8.3793	3.9163	3	.30—.20
11/31	7-2/28×1-16/27	29	9	2.1315			
12/31	7-2/28 selfed	43	12	2.6181			
	Total	**210**	**65**				
9/37	1-5-10/33 selfed	71	24	6.0631			
					7.3788	1	<.01
17/37	12-9/35×1-5-10/33	44	26	9.6570			
	Total	**115**	**50**				
	MALE HETEROZYGOUS BACKCROSSES						
19/36	2-3/32×2-8-6/32	107	40	—	56.1125	1	.01
	FEMALE HETEROZYGOUS BACKCROSSES						
1/29	L.G.×9-6-7/27	82	94	—	0.8181	1	.50—.30
13/31	3-33/29×2-52/29	126	123	—	0.0361	1	.90—.80
21/36	2-8-6/32×2-3/32	79	70	—	0.5436	1	.50—.30
28/36	2-8-5/32×2-7/32	58	34	—	6.2608	1	.02—.01
	Total	**345**	**321**	—	0.8648	1	.50—.30
	Heterogenity				6.7938	3	.10—.05

There is homogeneity within all the groups except one, but significant heterogeneity in the data taken as a whole. The logarithm likelihood expression for the data is

$$L = 104 \log(p) + 107 \log(1-p) + 534 \log(2-p).$$

On differentiating and equating to zero the value of p is $.2351 \pm .021$. Like the $T:t$ it is homogeneous; $\chi^2 = 1.9298$ for one degree of freedom, p = .20—.10.

The data supplying information on the crossover value between T and G are summarized in table 5. The heterogeneity χ^2 is on the margin of significance, but this is almost entirely due to the high percentage of crossing over in one family, $T\,g/t\,G$ selfed. There is no apparent cause for this abnormality, but it may be due to environmental changes. The families have not all been raised in the same year, consequently different temperature conditions may be present at the time of germ-cell formation.

TABLE 5

	TG	Tg	tG	tg	χ^2
$T\,G/t\,g$ selfed or intercrossed (10 families)	693	46	60	112	1.1926
$T\,g/t\,G$ selfed (1 family)	48	12	30	5	9.2759
$T\,G/t\,g \times t\,g/t\,g$ (1 family)	69	13	14	80	0.0070
$T\,g/t\,G \times t\,g/t\,g$ (1 family)	16	63	54	16	3.0185
$t\,g/t\,g \times T\,g/t\,G$ (1 family)	12	32	95	8	0.4806
$T\,G/t\,g \times t\,G/t\,g$ (1 family)	46	6	15	20	0.0120
$t\,G/t\,g \times T\,G/t\,g$ (1 family)	57	4	3	23	0.04819
D.F. = 6. p = .05—.02					14.4688

The crossover values for the three genes in percentages are:

$T\ G$	15.57 ± 1.0
$G\ W$	23.51 ± 2.1
$T\ W$	32.01 ± 1.5

The four linked genes $T\ G\ B\ X$ are all in the same group (LEWIS 1939) and the chromosome map, including the gene W, is given in figure 1.

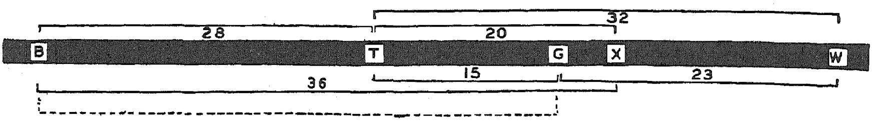

FIGURE 1.—Linkage map showing crossing over relations of five loci.

THREE POINT EXPERIMENTS

Three point backcross experiments have been analyzed to determine the coincidence value. The method of calculation devised by STEVENS (1936) has been followed. The crossover value for p and q and the coincidence value c may be calculated from the exact fit which is obtained in a two by two table (table 6). The coincidence value

$$c = \frac{T\,g\,W \times n}{(T\,g\,W + t\,G\,W) \times (T\,g\,W + t\,g\,W)}.$$

TABLE 6

$t\,g\,W/t\,g\,w \times T\,G\,W/t\,g\,w$

	Recombination in segment p	No recombination in segment p	
Recombination in segment q	$T\,g\,W$ $t\,G\,w$ (eliminated) pq	$t\,g\,W$ $T\,G\,w$ (eliminated) q(1−p)	q
No recombination in segment q	$t\,G\,W$ $T\,g\,w$ (eliminated) p(1−q)	$T\,G\,W$ $t\,g\,w$ (eliminated) (1−p)(1−q)	1−q
	p	(1−p)	1

The results of the two small three-point experiments are given in table 7.

TABLE 7

$t\,g\,W/t\,g\,w \times T\,g\,w/t\,G\,W$	$T\,G$	12	$p=0.136 \pm .028$
	$T\,g$	32	$q=0.272 \pm .036$
	$t\,G$	95	$c=1.470 \pm .223$
	$t\,g$	8	
$t\,g\,W/t\,g\,w \times T\,G\,W/t\,g\,w$	$T\,G$	54	$p=0.115 \pm .034$
	$T\,g$	4	$q=0.310 \pm .049$
	$t\,G$	6	$c=1.288 \pm .350$
	$t\,g$	23	

Both coincidence values are above unity, in one the difference is slightly more than twice the standard error and in the other the difference is less than the standard error. It is probable that the value does not differ significantly from unity. Had the genes *T G W* been situated much closer together, interference would have been expected and this would have given good evidence for the presence of the gene *W*. As this is not the case, large three point experiments were not undertaken.

DISCUSSION

Evidence has been given for a gene which either retards or inhibits pollen tube growth in the cultivated raspberry. This gene is linked with two marker genes *T* and *G*. No plants with *W* and *G* in the repulsed phase have, as yet, been found among the progeny of *T G W/t g w* plants selfed or intercrossed. However, the probability of finding a *g W/G w* plant among the 13 tested is about .2. This fact is therefore not incompatible with the general hypothesis that the disturbed ratios are caused by a linked gene *w* and not directly by the gene *g*. Evidence in support of this is given by family 28/36, *t t G g*×*T t g g*, in which there is a significant deficiency of *t* plants, thus showing that in this family a disturbed *T*:*t* ratio can be effected without the segregation of *G*:*g* on the male side. Conversely, there are four families in which a *T t G g* plant has been selfed and both *T*:*t* and *G*:*g* ratios do not exhibit any significant deviation from expectation. These results cannot be explained if the gene *g* is responsible for differential fertilization, but are explicable on the linked gene *w* hypothesis.

Similar cases of pollen tube genes causing differential fertilization have been found in maize, MANGELSDORF and JONES (1926), EMERSON (1934) and BURNHAM (1936), in which the effect is dependent upon the genetic constitution of the stylar tissue. However, the pollen tube genes induced by radium and X-ray treatment in Datura (BUCHHOLZ and BLAKESLEE 1936) are quite independent of the genetic constitution of the style. There is some evidence that the pollen tube gene *w* in raspberries behaves similarly to the pollen tube genes in maize. The raspberry variety Burnett Holme, *T t G G*, when selfed or backcrossed to its progeny always gives normal ratios for *T*:*t*. It is, therefore, assumed to be homozygous for *W*. Further, when Pyne's Royal (*W w*) or Lloyd George (*W w*) are crossed on to it, normal ratios for *T*:*t* are obtained (see table 1, families 9/36 and 10/36). The figures are small, and further data are necessary to confirm this.

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SUMMARY

A gene *w* causing differential fertilization in the cultivated raspberry is described. It causes aberrant ratios and heterogeneity in linked marker genes *T* and *G* when the particular genes are segregating on the male side.

The action of the pollen tube gene *w* is to inhibit completely, or to retard, pollen tube growth at some stage between pollen germination and fertilization. Crossover values between *W* and the marker genes *T* and *G* are calculated and a chromosome map with five genes is given.

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ADULT INTERMEDIATE-WINGED APHIDS NOT PHYSIOLOGICALLY INTERMEDIATE[1]

A. FRANKLIN SHULL
University of Michigan, Ann Arbor, Michigan

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CURRENT theory explains the production of structurally intermediate types of animals, including intersexes, by assuming that they start development as of one type, but change in some physiological respect early enough so that the later stages of development are those of a contrasted type. In the gypsy-moth intersexes of GOLDSCHMIDT, the reversal of the type of development was induced by the imbalance of strong and weak sex genes in hybrids. In aphids, the intermediates between parthenogenetic and gamic, and between winged and wingless individuals, have been produced under unusual combinations of light and temperature, applied at various times in the developmental cycle. The latter insects offer some advantages for the artificial production of intermediates, since the controlling agents may be applied at different times in different individuals.

In so far as intermediacy of aphids is expressed in chitinous structures, which can not change after the last molt, or in other structural features which, once established, change little or not at all, the intermediate condition of an adult may reflect only the processes and relations obtaining in the embryo or at latest in the nymphal stages. Heretofore these relatively changeless characters have been the chief if not the only ones used in judging the intermediacy of aphids. The principal possible exception to this generalization is found in the reproductive system, as involved in the intermediates between, or mosaics of, the gamic and parthenogenetic types. It is not known whether these irregular reproductive systems change during the adult life of the aphid or not; there would seem to be a possibility of such change, however. Color of the body, which is typically yellow in the gamic female, green in the parthenogenetic, may also change in the adult; but this color is so irregular that it has been little used in the classification of intermediates. In the intermediates between winged and wingless aphids, it appears possible that the wing muscles of the thorax may become modified during adult life. Some histological preparations by STILES (1938) show occasional signs of degeneration of these muscles, though none so extensive as to suggest that judgments regarding the grade of intermediacy would change in accord with them.

The other signs of intermediacy so far used are all such as are fixed at

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or before emergence from the fourth instar. The swollen hind tibia of the gamic female, or the half swollen one of an intermediate, remains of the same size from the time of its hardening after the last molt, and the number of sensoria in it is thereafter permanent. Even the depth of its brown color does not change. The wings of one of the parthenogenetic types remain for an indefinite time the same as at the conclusion of the last molt. An intermediate wing is thereafter of the same size; even its crumpled form is permanent after drying. The color of the long third segment of the antenna, and the number of sensoria in it, are permanent. The ocelli, if only partially developed in the young adult, do not change later; neither the cuticular lens nor the adjoining pigment experiences any later modification.

Under these circumstances, the criteria by which intermediacy has been judged practically all relate to things happening in the embryo or nymphal stages. If these characters develop in response to a physiological condition which at first determines a trend in one direction, but which is then replaced by or gradually modified to a condition which determines a trend in another direction, this replacement or modification is limited to the young stages. In the absence of more definite knowledge regarding changes in color of body, wing muscles, or reproductive system in the adult, intermediacy of the adult is a key only to developmental conditions.

It would be possible to suppose, therefore, that an *adult* aphid has completely emerged from the intermediate physiological conditions which made it an intermediate, and that it is then typically winged or typically wingless—except in those fixed structural characters which it acquired when it was physiologically intermediate. Indeed, the theory that intermediacy is due to having started development as of one type and finished it as of a contrasted type could lead to the conclusion that the adult ought to be definitely of one type—that type whose plan of development was followed in the later stages. Followed to its logical conclusion, the theory could even form a basis of judgment as to the direction in which the physiology of development had changed, provided the physiological nature of the adult can be ascertained; the direction of change would have to be toward the definitive adult type.

MEANS OF TESTING ADULT INTERMEDIACY

Fortunately for the ascertainment of the adult type in the contrast between winged and wingless aphids, there are two ways in which the nature of the adult, in other than structural respects, may be determined. In general, when the gamic phase of the cycle is reached, nearly all the males are born of wingless mothers, while nearly all the gamic females come from winged mothers. There are exceptions to this rule, but not many in most

strains. Furthermore, winged parthenogenetic aphids usually show a notably greater tendency to produce wingless offspring than wingless mothers do. These physiological distinctions can be used to detect intermediacy, or the lack of it, in adults at any age.

EARLIEST OBSERVATIONS ON ADULT PHYSIOLOGICAL INTERMEDIACY

In the work of this laboratory on the aphid *Macrosiphum solanifolii* the first opportunity to study the physiological properties of an adult aphid whose structural characters marked it as an intermediate was presented many years ago, in January 1927. Structural intermediates were rarely produced in the strain being reared at that time (clone A, SHULL 1932), and the experiments then performed were withheld from publication pending a fuller study of adult intermediacy. Then in the fall of 1929 clone A experienced a "mutation" in its behavior with respect to wings and the gamic phase (becoming clone A′, SHULL 1932). In this mutation the response to light in wing development was reversed and made much less definite, and very few gamic forms were thereafter produced. While structural intermediates in clone A′ became fairly common, their physiological properties with respect to wing production were less capable of definition, and with respect to gamic offspring seldom open to a test. Only gradually were the characteristics of another strain sufficiently discovered so that further tests of adult reactions in these two respects could be made. These comparisons have now been made and constitute the material of the later sections of this paper.

The intermediate aphid of 1927 was not the first intermediate obtained, but it was the first well marked one to appear in the midst of the gamic phase of the cycle. Abundant males were being produced by wingless mothers, many gamic females by winged mothers, and besides these numerous parthenogenetic females, of which the intermediate was one. Though the gamic phase was presumably half completed, there appeared still to be time for a moderate test as to whether the intermediate-winged aphid would produce males (like some of the wingless aphids), gamic females (like most of the winged ones), or both males and gamic females, or neither. Either of the latter two results, particularly both males and gamic females, could be regarded as a sign of intermediacy.

This adult intermediate had wings which were not quite flat (but not crumpled); which extended only slightly past the tip of the abdomen, hence were about three-fourths as long as normal wings; and which were not held in approximately vertical planes over the back as are typical wings. The long third segment of each antenna was about as dark as are those of winged aphids, and each bore 14 sensoria (as compared with usually 18 to 20 in typical winged females). The ocelli were well developed, not appreciably less so than those of winged females.

This female, intermediate with respect to wings and antennal sensoria though nearer to the winged type in both respects, and like the winged form with respect to ocelli, was reared in continuous light, since light appeared to have little to do with the production of gamic offspring. Her offspring were all gamic; the first ones were gamic females, then two males, and later additional gamic females. In all there were 11 gamic females, 2 males. This indicated an intermediate (or conceivably mosaic) adult physiology, leaning rather to the winged type, as did also her structural characters.

The control consisted of the numerous general experiments involving the same strain of aphids, in which winged mothers were producing chiefly gamic female offspring, while wingless mothers were producing parthenogenetic daughters (winged or wingless according to the light to which they were exposed) and usually some males toward the end of the family.

DIFFERENT GRADES OF STRUCTURAL INTERMEDIACY IN RELATION TO PHYSIOLOGICAL INTERMEDIACY

A generation later than the above, still in January 1927, three structural intermediates were obtained within a short period. One had wings a little less than half developed, one about one-fourth, while in the third the wings were mere protuberances. The first of these was preserved after producing a family, and was found to have rather dark antennae, one with 9 sensoria (as against usually not over 6 for wingless and not under 17 or 18 for winged), the other lost in preparing the balsam mount. Her ocelli were about half developed. This female leaned, therefore, slightly to the wingless side. The other two intermediates died unexpectly early, after producing only a few offspring, and their bodies could not be found; regarding their structural intermediacy, therefore, only the condition of the wings is known. From other intermediates it would be inferred that their ocelli would be but slightly developed, or not at all, while the antennae were probably nearly pale and presumably bore few if any more sensoria than the wingless females do.

Since the gamic phase of the cycle was by this time passing, there was no assurance that males would be obtained any more, even from wingless aphids. Accordingly, these intermediates were reared in alternating light (8 hours) and darkness (16 hours), so that a test of physiological intermediacy with respect to wing production would be made if the gamic offspring did not materialize. Definite controls (typical winged and wingless females) were reared, as nearly simultaneously with the intermediates as was possible in view of the fact that the three intermediates were not exactly contemporaneous. The progeny of all these aphids are shown in table 1.

TABLE 1

Progeny of structurally intermediate aphids as compared with those of typical winged and wingless parents.

CONDITION OF WINGS OF MOTHER	OFFSPRING			
	PARTHENOGENETIC		GAMIC	
	WINGLESS	WINGED	FEMALE	MALE
11 typical winged females	0	0	87	0
Less than half developed	10	12	0	0
About one-fourth developed	0	4	0	0
Mere protuberances	5	0	0	0
9 typical wingless	39	270	0	4

The physiological character of the typical winged females was unequivocal, they were producing only gamic female offspring. None of the intermediates was like them in this respect.

The typical wingless aphids produced only a few males, most of them none at all. Hence the absence of any males in the progeny of the three intermediates is scarcely proof that they were not typically wingless in their physiology. As for wing production, the most reasonable conclusion to be drawn was that the intermediates were physiologically as well as structurally intermediate. For, if wings may be regarded as independent of the reproductive system, the offspring of winged mothers were all wingless (the gamic females), the offspring of wingless mothers were mostly winged, while the offspring of the three intermediates were collectively nearly equally divided between winged and wingless. However, in view of the small number of offspring and of the irregularity of wing production in different families that "should" react alike, no reliance can be put on such a conclusion.

In general, it is clear that the three intermediates were not physiologically winged; they may have been intermediate or possibly wingless in their physiology.

The original aim in these early experiments, to distinguish among different grades of intermediacy, was foiled by the small number of offspring, particularly from the two parents with slighter wing development.

EXTENSIVE TESTS OF ADULT INTERMEDIACY

When the strain of aphids collected in Ann Arbor in 1931 had been sufficiently studied to show that winged and wingless adults produce dissimilar progenies, and that occasional intermediates appeared, a new set of tests of adult intermediacy was instituted. By that time, also, it had been demonstrated that the mutated clone A′, though less definite in its

responses, did nevertheless exhibit a difference between the progenies of winged and wingless parents. This latter strain had one advantage over that of 1931, in that it produced intermediates abundantly.

Many intermediates of both of these strains were reared under those conditions of light and temperature (not the same for both strains) which experiments had shown were most likely to produce differences between the families from winged and wingless parents (SHULL 1935). Controls from winged and wingless parents were raised at the same time under the same conditions. The aggregate offspring from all of these tests are shown in table 2.

TABLE 2

Progeny of intermediate-winged parents of two strains of aphids, compared with offspring of winged and wingless parents from the same strains.

STRAIN	PARENTS	OFFSPRING						
		TOTAL	PERCENTAGE					
			WINGLESS	WINGED	INTERM.-WINGED	GAMIC ♀	INTERM.-GAMIC-PARTH.	MALE
1931	Wingless	1618	42.9	53.2	2.2	0.0	0.0	1.7
	Interm.	2199	42.8	52.0	5.2	0.0	0.0	0.0
	Winged	946	62.2	18.1	1.9	17.8	0.1	0.0
Clone A′ (1923, 1929)	Wingless	2976	35.3	64.1	0.0	0.0	0.0	0.5
	Interm.	2146	22.1	73.1	0.1	0.0	0.0	4.8
	Winged	2037	59.0	41.0	0.0	0.0	0.0	0.0

Intermediates of 1931 strain

In the 1931 strain, wingless parents produced 53.2 percent winged offspring under the conditions of the experiments, winged parents only 18.1 percent of winged offspring. The intermediates reacted practically the same as the wingless (52.0 percent winged).

With respect to gamic female offspring, the winged parents produced 17.8 percent, wingless parents none. Here again the intermediates were like the wingless. Unfortunately the gamic phase was so little marked during these experiments that only 1.7 percent male offspring were produced even by wingless parents. This number does not provide a very sharp distinction between wingless and winged parents, hence the fact that the intermediate parents produced no males may not have much significance.

The number of intermediate-winged offspring is too nearly the same (2.2 percent from wingless parents, 1.9 percent from the winged) to provide a test. It is perhaps significant that the intermediate parents produced

more than twice as many intermediates (5.2 percent) as did either the winged or wingless, but whatever meaning it has is probably not related to the wingedness or winglessness of the adult intermediates in a physiological sense. This tendency for intermediates to produce intermediates is discussed elsewhere.

Intermediates of clone A′

In clone A′, the wingless parents produced 64.1 percent winged offspring, the winged parents only 41.0 percent winged offspring. This difference is in the same direction as in all other strains that have been tested, but is not so great as in the 1931 strain. It is one of the characters of the original 1923 strain which was not reversed in the mutation of 1929. Compared with these two percentages, intermediate-winged parents yielded 73.1 percent of winged offspring, more than either winged or wingless parents produced. Since this number is outside of the range between wingless and winged and nearer to the wingless extreme, it might be said that the intermediates are "outwinglessing" the wingless. Certainly they are not imitating the winged parents.

The production of so many males (4.8 percent) by the intermediates as against 0.5 percent by the wingless and none by the winged parents constitutes a puzzle. Are the intermediates here again exceeding the wingless in the wingless direction, or is excess of males due to an intermediate physiology? The entire clone A′ produced very few gamic forms, but happened to be doing so to a slight extent during part of the period of these experiments. In other strains the males are derived almost solely from wingless parents, and the little evidence here afforded indicates that the same is true of clone A′. On their face, the facts would seem to show that the behavior of the intermediates is far beyond the extreme of the wingless.

The other possibility should, however, be examined; that is, that male production is not a marked characteristic of wingless aphids, but the result of a degree of intermediacy. The fact that gamic females are nearly always wingless, the males without exception winged, suggests that wings and sex have in part the same physiological basis. Ignoring everything but this common basis, one might assume that the concentration of something ranged from one extreme at which gamic females are produced and wings inhibited, to another extreme at which males and wings are produced. Production of males could be imagined to result from a concentration somewhat short of the latter extreme. It would thus be a mildly intermediate condition. On such an assumption, the intermediates of clone A′ which produced more males than the wingless parents did, could be regarded as intermediate in their physiology, though presumably nearer the wingless extreme.

Two facts speak against this interpretation. They do not argue against the supposition that wings and sex may both be in some measure dependent on the concentration of one and the same substance; they are against the postulate that male production falls into an intermediate position in the scale of concentration. First, males are born as a rule only toward the end of a family. If male production depends on a less extreme concentration than wing production does, this lower concentration must often have been arrived at by reduction from a higher concentration earlier. But this higher concentration must have been reached by an increase in concentration, unless that concentration is highest at the beginning of the reproductive period. Were the latter true, the family ought in general to be wingless and gamic female at first, winged and male later, which is far from what actually happens. A peak concentration in the middle of the reproductive period, arrived at by increase from a lower one at the beginning, should, therefore, be preceded sometimes by males born early in the family. But they practically never are born then.

A second fact opposing the assumption that male production is a somewhat intermediate phenomenon is that no intersexes have been found. If males are produced by an intermediate concentration of something, it might be expected that the concentration would occasionally approach that which would yield females, and intersexes would result. As stated, no one appears ever to have seen an intersexual aphid.

While these two obstacles are not necessarily insurmountable, they suggest that male production by wingless parents is not due to an intermediate condition of any sort in these parents, but that it is an extreme situation reached only by some females at certain stages in the cycle, and then only toward the ends of their lives. If this conclusion is justified, the intermediate parents in clone A′ which produced 4.8 percent male offspring were more extreme than the wingless parents. Why they were more extreme is not explained, but the intermediates appear to be nearer the wingless type—farther from the winged forms than the wingless ones are.

DISCUSSION

On the whole, the results obtained aside from the brief tests in 1927, indicate that intermediate-winged aphids are, in their adult stage, physiologically not very different from wingless ones. When they differ from wingless aphids, they have mostly proved to be more extreme—that is, more different from winged aphids—than wingless ones are. It should be pointed out that these conclusions are drawn from mass experiments, in which no aphid has been tested singly. Since, however, the aggregate results from the intermediates agree so closely with those from the wingless aphids, no individual could deviate much from the wingless standard

without being balanced by others diverging equally in the opposite direction. Hence by all the standards used in previous work, adult intermediate aphids are approximately wingless physiologically. The first intermediate reared in 1927 may not be a real exception to this statement, for it was nearly winged. This means that it was of nearly winged physiology in embryonic stages. From this early winged condition it could progress toward the wingless condition and still be somewhat intermediate as an adult. Such an assumption would be particularly justifiable if the inhibition of wings in this individual were an unusually slow process at all stages.

If intermediacy is the result of a change of physiology in the period of development, and if there has been only one major trend in that change, the direction of change must be in each case from winged to wingless. Heretofore, on the basis of experimental and observational data, it has been held that the direction of change could be either winged to wingless, or wingless to winged.

The new conclusion is in harmony with the histological evidence (SHULL 1938) that all aphid embryos have wing rudiments, but that these are suppressed in the late embryo and first instar of those individuals which will be wingless. It is not in agreement with the conclusions of STILES (1938) who, after a histological study involving wing muscles and ocelli, believed that his observations indicated change in either direction in different individuals. One of his criteria was the nature of the wing-muscle cells, which in some individuals appeared necrotic as if a winged individual were losing its winged characters, in other individuals appeared to be actively continuing. This evidence seemed to be satisfactory.

How may the new results be reconciled with those of STILES? Since the physiology tested in these experiments is that of the adult, it is merely the end product of a series of events. It, therefore, represents only a major trend. The end result may have been reached by either a straight course or a devious one, just as a mountain peak may be reached over a railway whose incline is everywhere the same, or by a wagon road up and down over a succession of ever higher foothills. If it be supposed that the major change from winged to wingless in wingless aphids be a devious one, there would be periods in which the change would be in the opposite direction. During these temporary recessions could occur the active growth of the wing muscles and other processes indicating an approach toward the winged type instead of away from it.

The same assumption of an irregular, temporary reversible, approach to winglessness might also explain those fairly numerous intermediates whose combinations of distinguishing characters do not fit either a winged-to-wingless or a wingless-to-winged change.

If the direction of change, when there is a change, is always from winged

to wingless, it may be questioned whether, in the relation of gamic to parthenogenetic females, the change, when there is one, is not always from gamic to parthenogenetic. Some earlier experiments (SHULL 1930 a, b) indicated that winged mothers in the gamic phase of the cycle could easily be diverted (by high temperature) from the production of gamic daughters to production of parthenogenetic ones, but that the reverse change was impossible. That is, winged mothers in the parthenogenetic phase of the cycle could not be induced, by low temperature or any other tested agent, to produce gamic daughters.

In both of these changes there is a loss of certain characteristics. Wingless aphids lack the wings, wing muscles, ocelli, and some of the sensoria which winged ones have, and the only changes from the one type to the other appear to be in the direction of loss. Parthenogenetic aphids lack the colleterial glands, seminal receptacles, and the swelling and sensoria of the hind tibia of gamic females; the parthenogenetic germaria are smaller than those of the gamic; and the oocytes of the parthenogenetic lack the size, and yolk, and ability to undergo a second oogenetic division which the oocytes of the gamic females all possess. If change occurs only from gamic to parthenogenetic, again it involves only loss. It may well be that the reason why change occurs in one direction only is that inhibition of a developmental process in a structure determined for it in a labile fashion is possible, while stimulation of the same process in a structure having no such (even labile) determination is quite impossible. This would mean only that the necessary stimulating agent, because it is not the opposite of the known inhibiting agent, has not been discovered.

If the gamic-parthenogenetic intermediates are produced by change in only one direction, from gamic to parthenogenetic, it will be necessary to revise for them also the scheme by which the combinations of characters they possess are explained. It has been supposed that some arose by a change from gamic to parthenogetic, others by a change from parthenogenetic to gamic. After an order of determination of the distinguishing structures of the two kinds of females had been decided upon, the combinations of characters of the intermediates appeared to throw many of them into one or the other of the two classes arising from the two directions of change (SHULL 1933). But there were exceptions—many of them. It may now be necessary to assume that all the intermediates which do not fit the *one* direction of change owe their combinations of characters to fluctuations in the progress from gamic to parthenogenetic, as has just been suggested for the winged-wingless intermediates.

It is unfortunately not yet possible to test gamic-parthenogenetic intermediates to ascertain whether they may be physiologically parthenogenetic, for they do not reproduce, and no other physiological difference be-

tween gamic and parthenogenetic females has so far been established that could serve for that purpose.

Two rather incidental matters require further comment. One is the fact that in the 1931 strain (and to a very slight extent in clone A′) intermediate-winged parents produce more intermediate offspring than do either winged or wingless parents. There must be some holdover effect, the nature of which is not at present understood. It is probably related in some way to the tendency of winged parents to produce wingless offspring, and of wingless parents to produce winged offspring.

The other matter is the fact that intermediates of clone A′ exceeded, physiologically, the winglessness of wingless parents. This may be due to a slow start in the accumulation of the wing-inhibiting substance, compensated by a rapid increase of it later and therewith an excessive final concentration.

SUMMARY

Some observations in earlier experiments had suggested that adult intermediate-winged aphids might be also physiologically intermediate at that stage. Since their structural intermediacy is only a consequence of their intermediacy in the embryonic stages, and since intermediates presumably owe their condition to a change in the trend of their development, it seemed likely that the adult would no longer be intermediate. Or, if it were intermediate, some modification of the theory of the origin of intermediacy would be required.

A number of intermediates of two strains were tested as to their tendency to produce (1) winged offspring under certain circumstances, in which respect winged and wingless parents differ, and (2) gamic females (which typically come mostly from winged mothers) and males (mostly from wingless mothers). In one strain the intermediates were almost identical with the wingless aphids in both of these respects. In the other strain, the intermediates were more extreme (less like winged aphids) than the wingless parents were.

These results, indicating that structural intermediates are not physiologically intermediate, appear to fix the direction of change which produces intermediates as from winged to wingless. This is the direction of histological change, since all wingless individuals have wing rudiments in the embryo, only to lose them in the first instar.

The transition from winged to wingless is in all characters (wings, ocelli, wing muscles, antennal sensoria) a loss of parts. It thus appears possible to induce loss by environmental treatment; but the agent or agents which will stimulate development of new parts have not been discovered.

It is now suggested that gamic-parthenogenetic intermediates are also

produced by change in only one direction (gamic to parthenogenetic), which in most characters is obviously, and in the rest at least conceivably, also a loss of parts.

The proved conclusion regarding winged-wingless intermediates, and the suggested one concerning gamic-parthenogenetic intermediates, call for a developmental trend which changes its course in temporary fluctuations even though the general trend is always the same.

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TRANSLOCATIONS IN MAIZE INVOLVING CHROMOSOME 3

E. G. ANDERSON AND R. A. BRINK[1]
California Institute of Technology, Pasadena, California, and University of Wisconsin, Madison, Wisconsin

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INTRODUCTION

THE third longest chromosome of maize has the spindle attachment well away from the center, the long arm being twice the length of the short arm. It was shown to be associated with the a_1 ts_4 linkage group by means of deficiencies involving A_1 (McCLINTOCK 1931) and likewise by means of trisomics (McCLINTOCK, unpublished, cited by EMERSON, BEADLE, and FRASER 1935). This association is confirmed by the extensive series of translocation data summarized in the following pages.

ORIENTATION OF THE LINKAGE MAP

The A_1 deficiencies (McCLINTOCK 1931) involved the end of the long arm and indicated the orientation to be such that the a_1 end of the linkage map should lie toward the long arm and the *cr* end toward the short arm. This order has also been amply confirmed. Following the convention of orienting the maize linkage maps so that the left end corresponds to the short arm of the chromosome, the chromosome 3 map given by EMERSON, BEADLE and FRASER (1935) should be reversed to read as follows:

cr	d_1	*Rg*	ts_4	ba_1	*na*	a_1
0	18	40	47	64	75	103

TRANSLOCATIONS

The present study includes the following translocations which have been previously listed (ANDERSON 1935) with references to earlier literature: T1-3a, 1-3d, 2-3b, 2-3c, 2-3d, 3-5a, 3-5b, 3-5c, 3-6a, 3-6b, 3-7a, 3-7b, 3-8a, 3-8b, 3-9a, 3-9b, 3-10a, 3-10b, and 3-10c; also one translocation, T3-9c, described more recently (ANDERSON 1938). In addition two translocations not previously listed are included.

T2-3e was obtained from X-rayed seed (ANDERSON 1935 Lot 1). It gave two rings of four in intercrosses with T1-5b, 1-5c, 1-9a, 4-5a, 4-9b, 6-9a, and 8-10c, and a ring of six chromosomes with T1-2c, 2-4a, 2-9b, and 3-10a. The chromosomes involved have been checked by linkage tests.

[1] Contribution from the Division of Biology, California Institute of Technology and the Department of Genetics, University of Wisconsin (Paper No. 245). The junior author wishes to express his appreciation to the Wisconsin Alumni Research Foundation whose support during a leave of absence enabled him to participate in this work.

T3-7c was obtained by DR. L. J. STADLER, Columbia, Missouri, from an X-ray treated stock. It gave two rings of four in intercrosses with T1-2a, 4-5a, 4-8a, and 8-9a, and a ring of six chromosomes with T1-7a, 3-9a, 3-10a and 5-7a. The chromosomes involved have been checked also by linkage tests and by prophase cytology.

Some additional information has been published on T3-9a, 3-9b and 3-9c (ANDERSON 1938), on T3-8a and 3-8b (ANDERSON 1939) and on 1-3a (EMERSON 1939).

CYTOLOGY

The mid-prophase of meiosis has been observed in ten of the translocations studied. The positions of these translocations are recorded below in decimal fractions of the distance from the centromere (ANDERSON 1938). Thus 3L.1 indicates chromosome 3, long arm, one-tenth of the distance from the centromere to the end of the chromosome.

T1-3a	1S.2	3L.2
T2-3c	2S.6	3S.8
T3-6b	3S.8	6 satellite
T3-7a	3S.2	7L.25
T3-7b	3S.8	7L.1
T3-7c	3L.6	7L.5
T3-8a	3L.6	8L.8
T3-8b	3L.1	8L.2
T3-9c	3L.1	9L.2
T3-10a	3L.1	10L.1

The record on T3-6b is from CLARKE and ANDERSON (1934). The remainder are from preparations made by MRS. GERTRUDE G. FRANDSEN.

LINKAGE DATA

Backcross linkage tests have been made chiefly with the three gene combinations *na* a_1, ts_4a_1, and d_1lg_2. The order of four of these genes is d_1-ts_4-*na*-a_1, the three regions thus delimited being nearly equal in length and covering a total map distance of approximately 85 units. The data necessary to place lg_2 accurately in the series are not available, although two-point tests with d_1 and a_1, respectively, indicate that the gene may lie about half way between these loci. The map region which lg_2 serves to mark doubtless coincides in part with those of both ts_4 and *na*. (cf. EMERSON, BEADLE and FRASER 1935)

The data resulting from the linkage tests are presented in tables 1 to 6. The backcrosses involving *na* a_1 are given in tables 1 and 2, table 1 including the translocations to the left of *na*, table 2 those between *na* and a_1. Table 3 gives the backcrosses with ts_4a_1 where the order is definitely

T-ts_4-a_1 and table 4, those where the order is ts_4-T-a_1 and those where the translocation is near ts_4 but the order not clearly shown. Table 5

TABLE 1

Backcross progenies from $\frac{\text{T} \quad + \quad +}{+ \quad na \quad a_1}$.

TRANSLOCATION	PARENTAL COMBINATIONS		RECOMBINATIONS REGION 1		REGION 2		REGIONS 1, 2		TOTAL	PERCENT RECOMBINATION T-*na*	*na*-a_1
1-3a	80	63	9	14	27	25	0	6	224	12.9	25.9
2-3c	42	37	21	24	28	20	5	14	191	33.5	35.1
3-6a	71	66	31	38	51	29	11	27	324	33.0	36.4
3-7b	84	109	65	85	54	69	40	53	559	43.5	38.6
3-8a	101	98	16	10	56	43	4	9	337	11.6	33.2
3-8b	27	23	12	19	25	15	4	11	136	33.8	40.4
3-10a	58	58	15	35	30	30	16	13	255	31.0	34.9
3-10b	71	42	21	32	28	25	10	16	245	32.2	32.2

TABLE 2

Backcross progenies from $\frac{+ \quad \text{T} \quad +}{na \quad + \quad a_1}$.

TRANSLOCATION	PARENTAL COMBINATIONS		RECOMBINATIONS REGION 1		REGION 2		REGIONS 1, 2		TOTAL	PERCENT RECOMBINATION *na*-T	T-a_1
2-3d	90	55	15	11	4	6	1	0	182	14.8	6.0
2-3e	76	77	4	11	30	26	1	1	226	7.5	25.7
3-5b	147	169	8	6	40	18	1	4	393	4.8	16.0
3-5c	191	177	24	26	15	39	2	4	478	11.7	12.6

TABLE 3

Backcross progenies from $\frac{\text{T} \quad + \quad +}{+ \quad ts_4 \quad a_1}$.

TRANSLOCATION	PARENTAL COMBINATIONS		RECOMBINATIONS REGION 1		REGION 2		REGIONS 1, 2		TOTAL	PERCENT RECOMBINATION T-ts_4	*ts*-a_1
1-3d	18	6	5	7	21	17	4	5	83	25.3	56.6
2-3c	103	91	19	23	104	100	22	27	489	18.6	51.7
3-7b	91	111	28	41	86	101	31	46	535	27.3	49.3

gives the backcrosses with $d_1 lg_2$, where the order is d_1-T-lg_2 and those where the translocation is so close to d_1 that the order is uncertain. Table

TABLE 4

Backcross progenies from $\frac{+ \quad T \quad +}{ts_4 \quad + \quad a_1}$.

TRANS-LOCATION	PARENTAL COMBINATIONS		RECOMBINATIONS REGION 1		REGION 2		REGIONS 1, 2		TOTAL	PERCENT RECOMBINATION ts_4-T	T-a_1
2-3e	126	100	37	33	28	26	5	4	359	22.0	17.5
3-5b	89	79	49	36	17	28	17	13	328	35.1	22.9
3-9b	55	23	22	21	9	8	0	2	140	32.1	13.6
1-3a	100	107	2	5	111	66	4	6	401	4.2	46.6
2-3b	48	58	0	2	47	31	0	0	186	1.1	41.9
3-7a	48	41	2	4	49	52	1	3	200	5.0	52.5
3-7c	18	11	5	1	4	3	2	1	45	20.0	22.2
3-8a	239	200	9	0	123	104	2	6	683	2.5	34.4
3-9a	221	134	4	3	136	149	8	4	659	2.9	45.1
3-10a	56	81	5	6	61	60	15	4	288	10.4	48.6
3-10b	89	53	0	1	50	50	1	0	244	0.8	41.4
3-10c	181	144	2	1	85	124	0	1(?)	538	0.7	39.0

6 gives a single backcross where the order d_1-lg-T is indicated. Some further data involving backcrosses with a single gene will be presented in the discussion of the particular translocation involved.

TABLE 5

Backcross progenies from $\frac{+ \quad T \quad +}{d_1 \quad + \quad lg_2}$.

TRANS-LOCATION	PARENTAL COMBINATIONS		RECOMBINATIONS REGION 1		REGION 2		REGIONS 1, 2		TOTAL	PERCENT RECOMBINATION d_1-T	T-lg_2
1-3a	139	119	38	38	9	5	1	6	355	23.4	5.9
3-5a	53	41	17	17	6	5	0	0	139	24.5	7.9
3-6a	77	107	15	21	8	13	5	4	250	18.0	12.0
3-7a	119	100	30	26	23	19	9	1	327	20.2	15.9
3-8a	30	40	13	16	3	1	0	2	105	29.5	5.7
3-8b	37	32	7	7	4	2	1	1	91	17.6	8.8
3-9a	77	55	21	39	26	11	12	15	256	34.0	25.0
3-10a	80	52	10	7	20	9	0	2	180	10.6	17.2
1-3d	123	101	0	0	67	43	0	2	336	.6	33.3
2-3c	193	199	1	0	109	102	1	3	608	.8	35.4
3-7b	149	177	0	0	92	80	2	0	500	.4	34.8

TABLE 6

Backcross progeny from $\dfrac{+ \ + \ T}{d_1 \ lg_2 \ +}$.

TRANS-LOCATION	PARENTAL COMBINATIONS		RECOMBINATIONS REGION 1		REGION 2		REGIONS 1, 2		TOTAL	PERCENT RECOMBINATION d_1-lg_2	lg_2-T
3-9b	46	46	19	28	3	6	3	0	151	33.1	7.9

SUMMARY OF INFORMATION ON INDIVIDUAL TRANSLOCATIONS

T1-3a

For determination of linkage position, the data from tables 1, 4 and 5 may be summarized as follows:

Table 1	224 plants	T-12.9-na-25.9-a_1
Table 4	401 plants	ts_4-4.2-T-46.6-a_1
Table 5	355 plants	d_1-23.4-T-5.9=lg_2

The translocation lies between d_1 and the two genes lg_2 and na, closer to the latter. The order with reference to ts_4 is uncertain, but the relatively low crossing over between T and either lg_2 or na suggests as the probable order ts_4-T-lg_2. The observed cytological position is about L.2.

T1-3d

In addition to the backcrosses listed in tables 3 and 5, a backcross progeny of 170 plants involving only na gave 74 crossovers, or a percentage of 43.5. These data may be summarized as follows:

Table 3	83 plants	T-25.3-ts_4-56.6-a_1
Table 5	336 plants	d_1-0.6-T-33.3-lg_2
na	170 plants	T-43.5-na

The translocation is very close to d_1 near the left end of the map. The order with reference to d_1 is uncertain, but the data of table 5 favor the order T-d_1-ts_4.

T2-3a

This translocation is under investigation by DR. C. R. BURNHAM, Division of Agronomy and Plant Genetics, University of Minnesota, St. Paul. DR. BURNHAM kindly permits us to state that, according to his observations, the break in chromosome 3 is about midway between the centromere and the end of the long arm. Further unpublished results of genetic tests by DR. Burnham have given the following percentages of recombination: a_1-21.6; ts_4-15.1; na-10.4.

T2-3b

The data from table 4 show the translocation to be close to ts_4. The crossing over shown is 1.1 percent. In view of the long map distance between ts_4 and a_1, the data do not show whether the translocation is to the left or right of ts_4. Preliminary cytological observations indicate a great deal of non-homologous pairing, with the possibility that an additional chromosomal disturbance has occurred at or near the point of interchange.

T2-3c

The data from tables 1, 3, and 5 together with an additional backcross involving only ts_4 are as follows:

Table 1	191 plants	T-33.5-*na*-35.1-a_1
Table 3	489 plants	T-18.6-ts_4-51.7-a_1
Additional ts_4	81 plants	T-12.1-ts_4
Table 5	608 plants	d_1-0.8-T-35.4-lg_2

The translocation is very close to d_1, with the order uncertain. The data slightly favor the order T-d_1-*ts*.

T2-3d

The backcross data from table 2 are augmented by larger backcross progenies involving the genes *na* and a_1 separately.

Table	182 plants	*na*-14.8-T-6.0-a_1
Additional *na*	439 plants	*na*-12.3-T
Additional a_1	200 plants	T-8.0-a_1

The translocation is between *na* and a_1, being closer to a_1. Of the known chromosome 3 translocations, it is nearest to the right end of the chromosome map. An average of all backcrosses gives the map values, *na*-13.0-T-7.1-a_1

T2-3e

The data from tables 2 and 4 are as follows:

Table 2	226 plants	*na*-7.5-T-25.7-a_1
Table 4	359 plants	ts_4-22.0-T-17.5-a_1

The translocation is between *na* and a_1, being closer to *na*. The average crossover value for the T-a_1 interval is 20.7 percent.

T3-5a

The only linkage data available are those of table 5 showing the order to be d_1-24.5-T-7.9-lg_2.

T3-5b

The data from tables 2 and 4 are as follows:

Table 2	393 plants	na-4.8-T-16.0-a_1
Table 4	328 plants	ts_4-35.1-T-22.9-a_1

The translocation is between na and a_1, being closer to na. The average for the T-a_1 interval is 19.

T3-5c

The data given in table 2, based on a backcross progeny of 478 plants, give the following order: na-11.7-T.12.6-a_1. The translocation is about midway between na and a_1.

T3-6a

Data from tables 1 and 5 and from an additional backcross involving only a_1 are as follows:

Table 1	324 plants	T-33.0-na-36.4-a_1
Table 5	250 plants	d_1-18.0-T-12.0-lg_2
Additional a_1	151 plants	T-45.0-a_1

The translocation is in the middle portion of the section between d_1 and lg_2. The linkage relation to ts_4 is unknown.

T3-6b

Cytological observations by CLARKE and ANDERSON (1934) place the interchange well out on the short arm, at about S.8. Backcrosses with d_1 gave only one crossover among 219 plants. The translocation is very close to d_1.

T3-7a

The data from tables 4 and 5 are as follows:

Table 4	200 plants	ts_4-5.0-T-52.5-a_1
Table 5	327 plants	d_1-20.2-T-15.9-lg_2

The translocation is in the mid-region between d_1 and lg_2, a short distance from ts_4. The direction from ts_4 is uncertain.

T3-7b

The data from tables 1, 3 and 5 are as follows:

Table 1	559 plants	T-43.5-na-38.6-a_1
Table 3	535 plants	T-27.3-ts_4-49.3-a_1
Table 5	500 plants	d_1-0.4-T-43.8-lg_2

The translocation is very close to d_1. While the order is uncertain, the data from table 5 slightly favor T-d_1-ts_4. Cytological observation places the interchange well out on the short arm, at about S.8.

T3-7c

The small backcross family of 45 plants listed in table 4 indicates a position midway between ts_4 and a_1. The cytological position of the interchange is about halfway, or slightly more than half way, out on the long arm, (L.5 to L.6).

T3-8a

Data from tables 1, 4 and 5 are as follows:

Table 1	337 plants	T-11.6-na-33.2-a_1
Table 4	683 plants	ts_4-2.5-T-34.4-a_1
Table 5	105 plants	d_1-29.5-T-5.7-lg_2

The translocation is rather close to ts_4. The order with reference to ts_4 is uncertain, but the relatively low crossing over with na and lg_2 suggests the order ts_4-T-na. The cytological position is about L.6. The cytological preparations were among the earliest ones made, and are less reliable than those made more recently.

T3-8b

In addition to data from tables 1 and 5, there are available some data on backcrosses with ts_4 and with lg_2.

Table 1	136 plants	T-33.8-na-40.4-a_1
Table 5	91 plants	d_1-17.6-T-8.8-lg_2
Additional lg_2	199 plants	T-17.6-lg_2
Additional ts_4	264 plants	no crossovers

The translocation is very close to ts_4. The cytological position of the break is in the long arm, not far from the centromere (about L.1).

T3-9a

Data from tables 4 and 5 are as follows:

Table 4	659 plants	ts_4-2.9-T-45.1-a_1
Table 5	256 plants	d_1-34.0-T-25.0-lg_2

The translocation is near ts_4 but the order with reference to this locus is uncertain.

T3-9b

Besides the data in tables 4 and 6, there is one backcross progeny involving only a_1.

Table 4	140 plants	ts_4-32.1-T-13.6-a_1
Table 6	151 plants	d_1-33.1-lg_2-7.9-T
Additional a_1	155 plants	T-24.5-a_1

The translocation is probably to the right of lg_2 in the direction of a_1, but the data are not conclusive. A three-point test with na a_1 or lg_2 a_1 is needed.

T3-9c

No backcross data have as yet been obtained. A few small F_2 cultures from a cross with *na* a_1 showed a considerable amount of crossing over between T and *na*, indicating a position for the translocation well to the left of *na*. This is in agreement with the cytological placement of the break at L.1.

T3-10a

Data are available from tables 1, 4 and 5 from an additional backcross involving only d_1.

Table 1	255 plants	T-31.0-*na*-34.9-a_1
Table 4	288 plants	ts_4-10.4-T-48.6-a_1
Table 5	180 plants	d_1-10.6-T-17.2-lg_2
Additional d_1	71 plants	d_1-12.7-T

The data are somewhat conflicting, but indicate the position of the translocation as nearest to ts_4. The data from table 4 favor d_1-T-ts_4 as the most probable order. The cytological position of the break is recorded as L.1 to L.2.

T3-10b

Data from tables 1 and 4 are as follows:

Table 1	245 plants	T-32.2-*na*-32.2-a_1
Table 4	244 plants	ts_4-0.8-T-41.1-a_1

The translocation is very close to ts_4.

T3-10c

The backcross data on 538 plants listed in table 4 show 0.7 percent crossing over with ts_4 and 39.0 percent with a_1.

THE DISTRIBUTION OF THE TRANSLOCATIONS ON THE CHROMOSOME

The translocations involving chromosome 3 fall into three groups on the basis of present data: (1) a group of four at the left end rather closely linked to d_1 (2) a group of five or six at the right end in the vicinity of *na* and a_1 and (3) the remaining translocations in the whole middle region of the chromosome map which show fairly close linkage with ts_4.

The group of translocations at the left end may be tabulated as follows:

	Cytological position	Crossing over with d_1	Crossing over with ts_4
T3-6b	S.8	0.5	
3-7b	S.8	0.4	27.3
2-3c	S.8	0.8	17.7
1-3d		0.6	25.3

In each of these the crossing over with d_1 is less than one percent. The three which have been studied cytologically have the interchange well out toward the end of the short arm of the chromosome.

The group at the right end may be tabulated in similar fashion:

T3-7c	ts_4-20.0-T-22.2-a_1
3-9b	lg_2- 7.9-T-18.0-a_1
3-5b	na- 4.8-T-19.1-a_1
2-3e	na- 7.5-T-20.7-a_1
3-5c	na-11.7-T-12.6-a_1
2-3d	na-13.0-T- 7.1-a_1

Only one of these, T3-7c, has been studied cytologically, its position being about L.6. But here the linkage data are so few that they can only be taken as indicating the position of the translocation in the general neighborhood of lg_2 and *na*.

The remaining translocations form a group rather closely linked to ts_4. The direction from ts_4 in each case is uncertain due to the long distance between the two genes ts_4 and a_1 used in the tests. Those tested with d_1lg_2 lie within this long interval, well away from either gene. Most of the translocations appear to partially suppress crossing over. These translocations are listed in table 7, arranged roughly in the order of the observed crossing over with d_1 and lg_2, where such data are available.

TABLE 7

Translocations closely linked to ts_4.

TRANS-LOCATION	CYTOLOGICAL POSITION	PERCENT CROSSING OVER WITH			
		d_1	lg_2	ts_4	na
T3-9a		34.0	25.0	2.9	
3-7a	S.2	20.2	15.9	5.0	
3-8b	L.1	17.6	14.8	0	33.8
3-9c	L.1				
3-10a	L.1	11.2	17.2	10.4	31.0
2-3b				1.1	
3-10b				0.8	32.2
3-10c				0.7	
3-6a		18.0	12.0		33.0
3-5a		24.5	7.9		
1-3a	L.2	23.4	5.9	4.2	12.9
3-8a	L.6	29.5	5.7	2.5	11.6

The apparent grouping of more than half of the translocations close to ts_4 is probably due in part to a partial suppression of crossing over in at least some of the translocations. It is also suggested that there may be a

considerable part of the middle portion of the chromosome in which relatively little crossing over takes place.

The centromere cannot yet be located with any certainty, except that its position must lie somewhere in the long region between d_1 on the left and the two genes lg_2 and na on the right. The most probable location is near ts_4. Closer determination must await further cytological study and linkage tests with homozygous translocations.

SUMMARY

Studies are reported on 21 translocations in maize involving chromosome 3. The translocations are distributed from the neighborhood of d_1, well out on the short arm, almost to a_1 in the distal part of the long arm.

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SOME EFFECTS OF COLCHICINE ON HEREDITY IN DAPHNIA LONGISPINA[1]

H. HOWARD DUNHAM AND A. M. BANTA
Brown University, Providence, Rhode Island

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INTRODUCTION

THE success of BLAKESLEE and AVERY (1937) and of NEBEL and RUTTLE (1938) in inducing polyploidy in plants by means of colchicine treatments has been followed by investigations of other plant materials by a number of workers (EIGSTI 1938, WALKER 1938, DERMEN 1939, LEVAN 1938, and RASMUSSON and LEVAN 1939). While polyploid plants have been obtained by treating diploid plants with colchicine, apparently no workers with animal materials have reported the production of polyploid animal forms by the use of the drug. Naturally occurring polyploidy is rare in animals but quite common among plants. DOBZHANSKY (1937, p. 219) states that "the prevalence of the polyploid series of chromosome numbers in plants and their relative scarcity among animals constitutes the greatest known difference between the evolutionary patterns in the two kingdoms."

During the summer of 1937 the authors became interested in the problem of treating the parthenogenetic eggs of a cladoceran with colchicine. It was hoped that colchicine might produce results in the developing diploid parthenogenetic egg of a cladoceran that would be comparable with results already obtained in plants. It seemed theoretically possible to induce tetraploidy in the developing parthenogenetic egg and thereby obtain a tetraploid clone of Cladocera. Presumably a tetraploid cladoceran, if obtained, could readily be propagated by parthenogenesis. Tetraploid or polyploid bisexual animals, for example Drosophila, have been shown to yield many sterile intersexes among their offspring—a fact commonly explained by the chromosome balance theory of sex determination (BRIDGES 1925).

The authors acknowledge the assistance of MISS RAE WHITNEY in preparing camera lucida drawings and in making cytological studies of some of the material. The technical advice of DR. THELMA R. WOOD on certain points of procedure is also acknowledged. MR. MALCOLM REID collaborated during the early part of the work.

MATERIALS AND METHODS

In all the experiments with colchicine to be reported in this paper a single clone of *Daphnia longispina* was used. This clone (Banta's Line

[1] Submitted by H. HOWARD DUNHAM in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Brown University, October, 1939.

1284) has been studied thoroughly in this laboratory and certain highly reliable norms have been established as regards instar duration, average numbers of young produced, growth, and duration of life (BANTA 1939).

In parthenogenetic reproduction, *D. longispina* mothers carry the developing young in a brood chamber until they are capable of entering upon a free-swimming existence at which time they are released ("birth"). The newly released young (ordinarily females) pass through three successive molts each of which marks the end of a juvenile instar. During the fourth (adolescent) instar the first clutch of parthenogenetic eggs appears in the ovaries. At the beginning of the fifth (first adult) instar the first clutch of eggs passes into the brood chamber. During the fifth instar these eggs develop in the brood chamber into young capable of a free-swimming existence and are released shortly before the mother molts the fifth time. The mother normally passes through 12 to 16 more instars before death, producing in each instar a brood of young from parthenogenetic eggs.

Manure infusion culture medium (BANTA 1921) was used throughout. In the nine series of colchicine treatments of the parthenogenetic eggs the proper amounts of a stock solution of colchicine were added to a given volume of the culture medium to yield a solution of the desired concentration of colchicine in culture medium. In the first three series of colchicine treatments young females in the fourth (adolescent) instar were placed in colchicine medium a few hours before the first clutch of eggs was to enter the brood chamber (beginning of fifth instar). In these three series the mothers were removed to culture medium without colchicine two hours after the eggs entered the brood chamber. In all later experiments each mother was placed in colchicine culture medium immediately after a clutch of parthenogenetic eggs had entered the brood chamber. The brood chamber is open to the surrounding medium so that eggs in the brood chamber were exposed to the colchicine in the culture medium.

Controls (untreated) were included in every series. All young obtained from colchicine-treated eggs and all young from sister control mothers in every series were isolated into separate bottles of culture medium at the time of their release and were reared until adult or until death. Every animal obtained from either colchicine-treated eggs or from control mothers was studied carefully for at least one or more adult instars or until death. Any individuals which did not appear quite typical morphologically or normal as regards growth and reproduction were given especially close attention. In nearly all cases young from apparently atypical animals were studied to determine whether the apparently abnormal characteristics were inheritable. Thus the general method of procedure was to attempt to discover any possible mutants among either the control or colchicine-treated groups.

TREATMENTS

The essential features of all nine of the series of treatments of the eggs are set forth in table 1. The treatments used are briefly outlined in the two left-hand columns. The three columns at the right in table 1 refer to the offspring developed from either treated or control eggs which were of especial interest to this study. Thus in Series I among 127 animals obtained from eggs treated for from seven to eleven hours at a concentration of 1/1,000,000 parts of colchicine by weight ten were sterile, producing no eggs. None of these 127 fell into the second category of animals which produced eggs that failed to develop. At the extreme right of the table is a column wherein are recorded cases of mutant clones obtained. One of the 127 animals under consideration gave rise to young by parthenogenesis which were distinctly different from the normal clone (Line 1284) and established a mutant clone (Line 49).

Series II and III yielded no animals which served to establish mutant clones. However, in each there were obtained several animals from treated eggs which either produced no eggs or produced varying numbers of parthenogenetic eggs which failed to develop or only partially developed. In either case it might be that mutations were involved which served to render the animals sterile and prevented their producing any eggs or, in individuals which produced eggs, prevented development of the eggs.

It will be noted that certain series of experiments yielded no results of any interest while from other series there were obtained considerable numbers of atypical offspring from treated eggs. Series V was, in certain respects, the most successful of all in that a total of 20 peculiar individuals were obtained among a group of 147 young from eggs treated at a concentration of 3/400,000 for one and one-half hours. It is interesting to note that among 167 animals from eggs treated simultaneously at the same concentration but for 20 minutes less time no unusual individuals appeared.

The treatments had to be carried out at room temperature which generally varied not more than one degree during the course of one series of treatments. Between different series, however, the temperature variation was much greater. In Series VI the average temperature was 23.5°C while in Series VII it was close to 17.0°C. Equal concentrations and periods of treatment in these two series gave very different results. In Series VI only two of approximately 180 treated eggs developed while in Series VII almost none of the treated eggs failed to develop. These two series represent extremes with respect to temperature and demonstrate that the toxicity of the drug toward the eggs is considerably increased at higher temperatures.

The totals at the end of table 1 afford a brief summary of the nine series

TABLE I

Summary of results of colchicine treatment on Daphnia longispina, Series I–IX.

In Series I–III treatments were begun before egg laying and continued two hours after the eggs were in the brood chamber.

In Series IV to IX treatments were begun promptly at egg laying and continued for the periods indicated in the table.

CONCENTRATION PARTS BY WT.	DURATION OF TREATMENT (HRS.)	NUMBER OF MOTHERS	YOUNG PRODUCED	PECULIAR ANIMALS FROM TREATED EGGS		
				PRODUCED NO EGGS	PRODUCED EGGS; NO YOUNG	MUTANT CLONES OBTAINED
			Series I. (10/11/37)			
1/1,000,000	7–11	12	127	10	0	1
1/10,000,000	9–11	12	144	1	0	0
Controls	—	5	69	0	0	0
			Series II. (11/14/37)			
1/10,000	$8\frac{1}{2}$–$16\frac{1}{2}$	38	0	—	—	—
1/100,000	5–14	29	0	—	—	—
1/1,000,000	$9\frac{1}{2}$–$13\frac{1}{2}$	20	286	5	5	0
Controls	—	5	81	0	0	0
			Series III. (11/30/37)			
1/100,000	6–8	5	0	—	—	—
1/200,000	6–11	4	0	—	—	—
1/400,000	9–13	5	24	1	0	0
1/600,000	9–12	5	65	3	2	0
1/800,000	7–11	5	65	1	0	0
1/1,000,000	6–11	5	66	0	0	0
Controls	—	2	28	0	0	0
			Series IV. (2/13/38)			
1/100,000	$\frac{1}{2}$–1	8	62	0	0	0
3/400,000	$1\frac{1}{2}$	1	14	0	0	0
Controls	—	1	12	0	0	0
			Series V. (2/27/38)			
3/400,000	$1\frac{1}{2}$	6	147	8	8	4
3/400,000	$1\frac{1}{6}$	6	167	0	0	0
Controls	—	2	58	0	0	0
			Series VI. (8/24/38)			
3/400,000	$1\frac{1}{2}$	9	2	0	0	0
Controls	—	3	32	0	0	0
			Series VII. (9/6/38)			
3/400,000	$1\frac{1}{2}$	10	130	0	0	0
Controls	—	3	36	0	0	0
			Series VIII. (11/4/38)			
3/400,000	$1\frac{1}{2}$	10	192	0	0	0
Controls	—	2	40	0	0	0

TABLE 1—*Continued*

CONCENTRATION PARTS BY WT.	DURATION OF TREATMENT (HRS.)	NUMBER OF MOTHERS	YOUNG PRODUCED	PECULIAR ANIMALS FROM TREATED EGGS		
				PRODUCED NO EGGS	PRODUCED EGGS; NO YOUNG	MUTANT CLONES OBTAINED
			Series IX. (1/13/39)			
1/100,000	$1\frac{1}{2}$	4	26	2	1	1
1/100,000	$1\frac{1}{4}$	3	58	0	1	0
1/100,000	1	3	47	1	1	0
3/400,000	$1\frac{1}{2}$	6	126	1	2	1
Controls	—	5	113	0	0	0
			Totals			
Treated	—	206	1748	33	20	7
Controls	—	28	469	0	0	0

of experiments. Of 1748 animals hatched from treated eggs 33 produced no eggs at any time in life. Moreover, among these none showed any microscopically detectable signs of ovarian activity at any time (repeated examination of the live animals). The second category is rather arbitrary. It includes all animals (20 from treated eggs) which produced some parthenogenetic eggs none of which developed. Some of these 20 produced nearly as many eggs as normal animals while others produced few (two to five eggs). While the 33 which produced no eggs and the 20 which produced eggs incapable of development were all sterile in effect, for convenience the two groups are considered separately.

Seven stem mothers which gave rise to mutant clones were obtained from among the 1748 animals from treated eggs. Each mutant clone so obtained was arbitrarily assigned a line number for purposes of convenience. Series I yielded the mutant clone, Line 49. From Series V were obtained Lines 86, 123, 159 and 171. Series IX produced the mutant clones, Lines 5 and 146.

MORPHOLOGICAL PECULIARITIES OF THE MUTANT CLONES

Some of the seven mutant clones exhibited certain morphological features that were more or less peculiar to these clones. However, in each clone exhibiting such morphological peculiarities there was a wide range of variability in degree of manifestation of the unusual feature. Simple body outlines drawn by camera lucida serve to illustrate some of the abnormal tendencies exhibited by some lines. Figure 1 shows an animal of the normal parent clone (Line 1284). Figure 2 shows an animal of Line 49 with a pronounced tendency for the body proper to project beyond the posterior margins of the valves of the carapace. This abnormal tendency

was also shown by animals of Lines 86 and 159 and rarely by individuals of Line 123. In these four lines there was also a tendency among the animals to exhibit a gap between the beak and the anterior margins of the valves of the carapace. This latter peculiarity occurred especially frequently among animals of Line 123 (see figure 3).

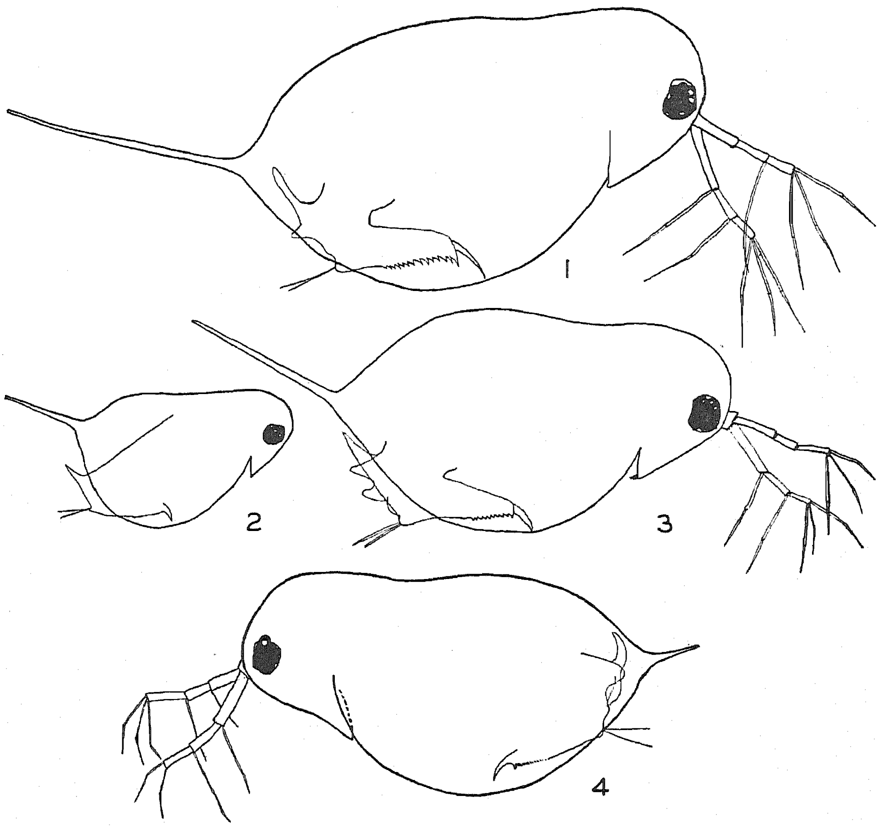


FIGURE 1.—Camera lucida outline of a normal animal of Line 1284. Age 10 days; actual body length 2.41 mm.

FIGURE 2.—Projection drawing of the outline of an animal of Line 49 (antennae omitted). Age 9.5 days; instar and body length not recorded.

FIGURE 3.—Camera lucida outline of an animal of Line 123; 8th instar. Actual body length 2.00 mm.

FIGURE 4.—Camera lucida outline of an animal of Line 146. Age 27 days; actual body length 2.06 mm.

Animals of Line 171 were extremely dwarfish but otherwise showed no morphological peculiarities. Line 5 was represented by only five animals produced by the stem mother of the clone. None of these five animals of the first parthenogenetic generation attained maturity. Figure 4 is an

outline of an animal of Line 146. The posterior margin of the beak in these animals characteristically lay between the anterior margins of the valves of the carapace. This peculiarity was interesting in that it was the opposite of the gaping condition already described which occurred in animals of Lines 123, 49, 86, and 159.

PHYSIOLOGICAL CHARACTERISTICS OF THE CLONES

During April and May, 1938, groups of animals from each of the four mutant clones (49, 86, 123, 171) were simultaneously studied from birth to old age and death in comparison with a control group of animals of Line 1284, the parent clone. In Line 49 the 16th parthenogenetic generation was used; in Line 86 the third; in Line 123 the fourth; and in Line 171 the second and third parthogenetic generations. All animals used in this study were within two days of the same age.

The numbers of animals of each line involved in this study are listed in table 2. Greater numbers were used for each mutant clone than for

TABLE 2

Average longevity (days) of four mutant lines compared with parent clone (Line 1284). Spring 1938.

GROUP	NO. STARTED IN GROUP	AV. FOR ENTIRE GROUP	NO. IN GROUP REACHING MATURITY	AV. FOR ANIMALS REACHING MATURITY
1284	25	35.8	25	35.8
123	43	13.3	25	21.1
86	47	15.2	39	17.7
49	41	10.4	22	15.8
171	48	6.3	12	19.1

Percent surviving given number of days for the above five groups

Line	5 days	10 days	15 days	20 days	25 days	30 days	35 days	40 days	45 days
1284	100%	100%	100%	96%	96%	80%	56%	28%	16%
123	60.5	58.1	51.2	39.5	11.6	0.0			
86	80.9	72.3	53.2	34.0	8.5	0.0			
49	58.5	46.3	34.1	4.9	4.9	2.4	0.0		
171	25.0	18.8	16.7	12.5	4.1	0.0			

controls because of the lower viability characteristic of all mutant clones. The data included in table 2 reveal that average longevity for each mutant clone was distinctly below that of the controls (Line 1284). Considerable numbers of animals in each mutant clone failed to reach maturity. In the lower part of table 2 it is shown that few or none of the animals in the mutant clones lived for 30 days. Eighty percent of the controls (Line 1284) lived beyond 30 days.

Curves representing growth (fig. 5) and reproductive data (fig. 6) clearly

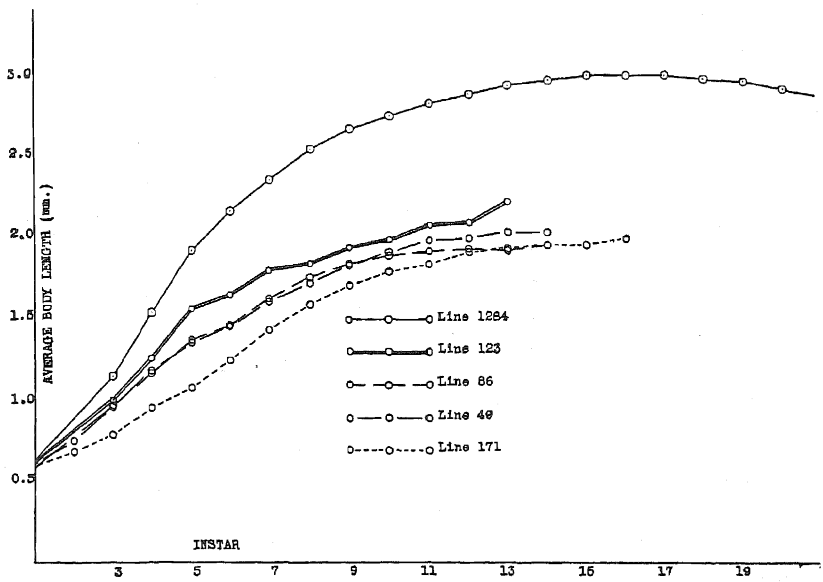


FIGURE 5.—Growth curves for Lines 49, 86, 123, 171 and 1284 (Spring 1938).

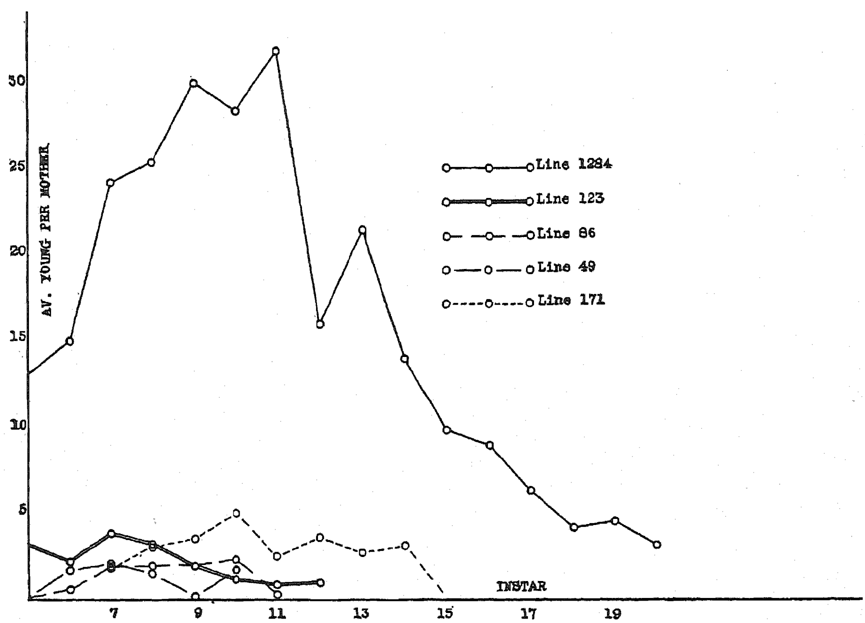


FIGURE 6.—Average numbers of young per mother each instar: Lines 49, 86, 123, 171 and 1284 (Spring 1938).

demonstrate the great differences between the normal parent clone (Line 1284) and the mutant clones. While these two figures require no further explanation it is of interest to note the practical identity of the growth curves for Lines 49 and 86. This is especially noteworthy in connection with the fact that animals of Lines 49 and 86 were morphologically indistinguishable. Line 171 was the most inferior as regards growth as it was in general viability. In figure 6 it appears that none of the animals of Line 171 reproduced until in the seventh instar whereas the other mutant animals of each line (a few at least) began to reproduce in the fifth instar, which is normal for *D. longispina*.

Line 123 was induced to reproduce sexually by means of a crowding technique described by WOOD (1932, 1938) and by WOOD and BANTA (1932) in order to study biparental inheritance in this clone. When Line 123 was thus inbred none of the forty-two fertilized sexual eggs obtained could be induced to hatch. Matings of females of Line 123 with normal males of Line 1284 resulted in sexual eggs a small percentage of which could be hatched. A similar small percentage of sexual eggs obtained from normal females of Line 1284 mated with males of the mutant Line 123 hatched. Individuals hatching from either type of mating were distinctly not normal as compared with Line 1284.

REVERSION TO NORMAL IN CERTAIN MUTANT CLONES

At the beginning of the summer of 1938 four mutant clones derived from Line 1284 by colchicine treatments were being maintained. These were Lines 49, 86, 123, and 171. In stock cultures of these clones 20 or more animals of each were reared in each generation. Generally the first few parthenogenetic females to reproduce in each generation served as the mothers of the next generation. Less detailed attention was given these clones during the summer than they received during their active study.

In July one young mother in the 24th parthenogenetic generation of Line 49 produced 12 young in her first brood. This was an abnormally large brood for an animal of this clone, being about normal for a first brood in Line 1284. Nevertheless these 12 young were (unwisely) used as members of the 25th generation of the clone. When these 12 had reached maturity it was realized that they resembled animals of Line 1284 more closely than they did animals of Line 49. In other words the mother of these 12 presumably was either normal in all respects herself or at least gave rise to normal young. The fact that the mother of these 12 young produced such a large brood suggests that she was normal. In this case Line 49 (in the mutant condition) was lost because all animals of the mutant stock had been discarded or were no longer producing young when this reversion to normal was recognized.

Line 86 (as a mutant clone) was likewise lost about two weeks later because of a similar reversion to normal that appears to have occurred in this clone in either the 13th or 14th parthenogenetic generation. The stocks of Lines 49 and 86 after their apparent reversion to normal were kept for a number of generations and continued to appear normal (like Line 1284) in all respects. Finally in October and November, 1938, 50 animals of Line 1284 were studied in parallel with a group of 40 animals of Line 49 and with a group of 50 of Line 86. The animals of Line 49 in this study were of the 36th parthenogenetic generation after the origin of the line; and those of Line 86 were of the 24th generation. This study was

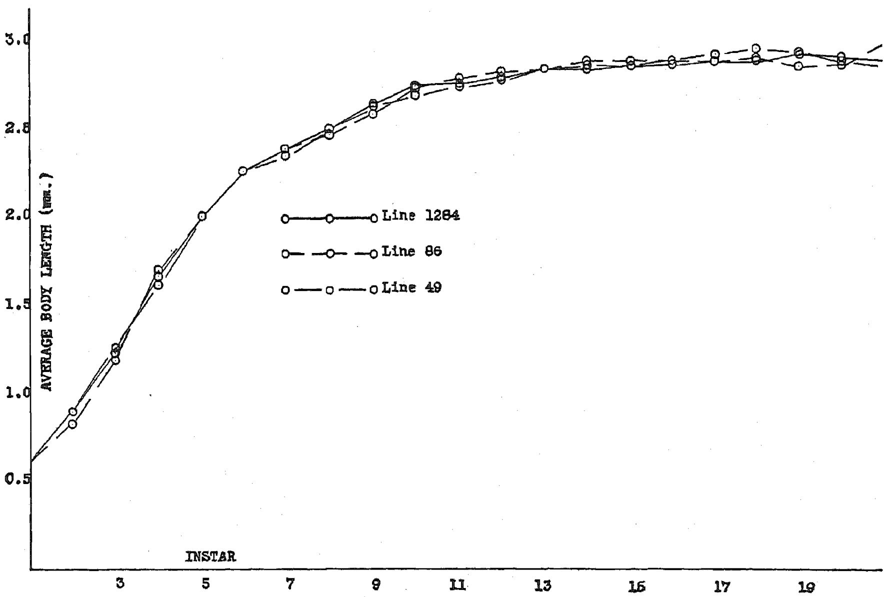


FIGURE 7.—Growth curves for Lines 49, 86, and 1284 after reversion to normal of Lines 49 and 86.

made in order to determine whether the apparent reversion to normal in the two formerly mutant clones had been complete. Data for growth and reproduction of these three clones so compared are presented graphically in figures 7 and 8. The three clones were indistinguishable in all respects so that it appeared that Lines 49 and 86 had reverted completely to the normal condition after 24 and 14 generations respectively as distinct mutant cones. Comparison of the curves for growth (fig. 5) and for reproduction (fig. 6) for Lines 49 and 86 in the mutant state with figures 7 and 8 reveal how striking had been the change in both mutant clones when the reversion to normal occurred.

Line 171 failed to survive the summer of 1938. The last parthenogenetic

generation of this clone that could be obtained was the ninth. Line 123 continued through the summer of 1938 in the mutant condition. However, in November, 1938, one animal of the 29th parthenogenetic generation of this line appeared to be normal and gave rise to what appeared to be normal offspring. All other individuals of the 29th generation of Line 123 were typical mutant individuals. By rearing successive generations from the one apparently normal animal which occurred in this line a normal sub-

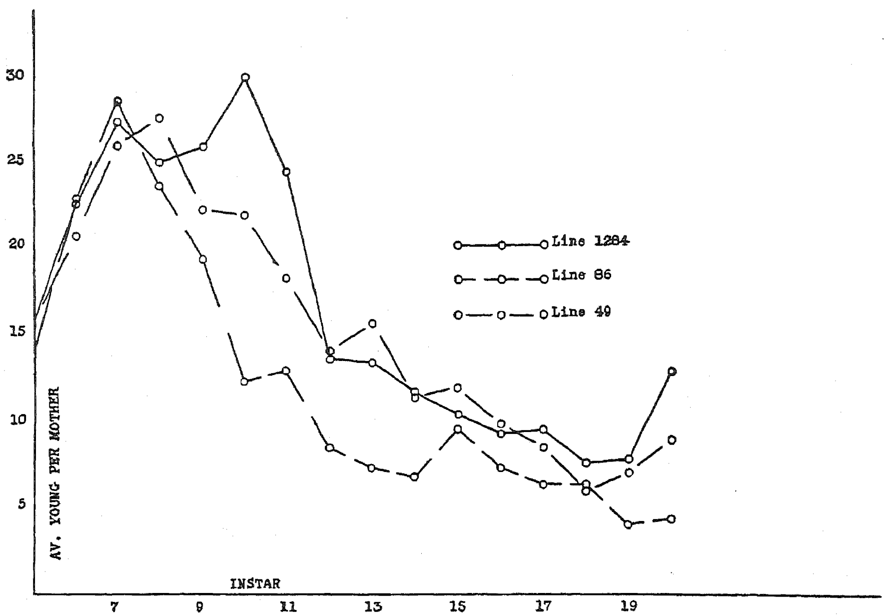


FIGURE 8.—Average numbers of young per mother each instar (Lines 49, 86, and 1284) after reversion to normal in Lines 49 and 86.

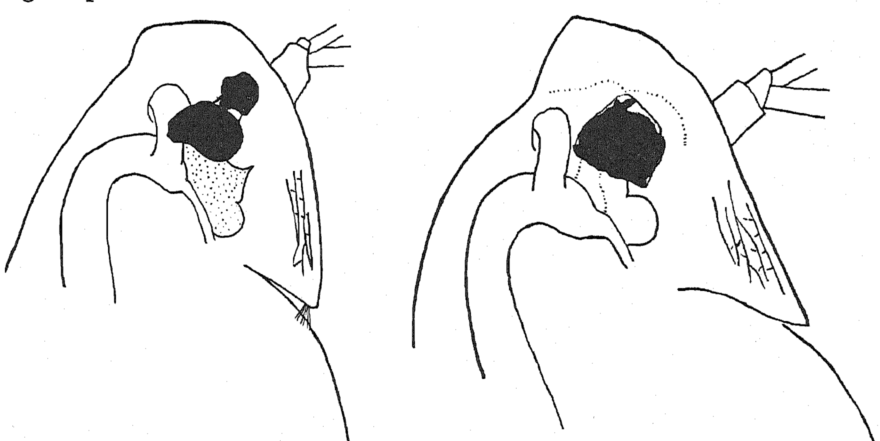
clone of Line 123 was established and was propagated along with the mutant Line 123. A study of this apparently normal sub-clone of Line 123 revealed that it was indistinguishable from Line 1284. In other words a complete reversion to normal was observed in Line 123 as had already happened in Lines 49 and 86. Mutant Line 123 is still being maintained after having passed through more than sixty parthenogenetic generations without noticeable change, except for the one case of reversion to normal just noted.

SPONTANEOUS MUTATION IN LINE 1284

No animal hatching from untreated eggs (controls) in the several series of colchicine treatments appeared in any respect significantly atypical for members of the normal clone (Line 1284). Line 1284 has been studied very

extensively in this laboratory but until now no spontaneous mutation has appeared which manifested itself phenotypically in parthenogenetic reproduction. BANTA (1939) has described spontaneous mutations occurring in other clones of Cladocera and has shown how recessive, spontaneous mutations arise and accumulate during parthenogenetic reproduction in these clones. These recessive mutations are made evident only when allowed to become homozygous during sexual reproduction.

In a group of control animals used for comparison of Line 1284 with Lines 49 and 86 after the reversion to normal in the latter two lines, there was one atypical, but supposedly genetically normal, individual. By parthenogenesis this female gave rise to only a few young which were reared for study and found to be highly abnormal in a number of respects. By parthenogenetic reproduction this newly derived mutant clone (Line 89) was propagated for five successive generations but could not be maintained longer. All animals of this clone except the stem mother grew at a subnormal rate and all, including the stem mother, were inferior in reproductive capacity. Morphological peculiarities of the individuals included irregular body outline (wrinkling of carapace) and the highly abnormal lobed appearance of the eyes exhibited by the majority of the clone. Figure 1 of a normal animal and figures 9 and 10 serve to illustrate these morphological peculiarities.



9 10

FIGURE 9.—Camera lucida outline of the head of an animal of Line 89 showing two pigmented lobes of the eyes. Age of animal, 7 days.

FIGURE 10.—Camera lucida outline of the head of another animal of Line 89 showing less extreme irregularity of the eye. Age of animal, 4 days.

CYTOLOGICAL STUDY OF LINE 123

MISS RAE WHITNEY, in this laboratory, made acetocarmine smear preparations of embryos of Line 123 (mutant) and of the sub-clone of Line

123 which had reverted to normal, and compared the cytological picture obtained with that revealed in a study of the embryos of Line 1284. MISS WHITNEY found chromosome counts extremely difficult in this material but found the diploid number to be between 12 and 14 in the mutant and normal Line 123 and in Line 1284. It seems possible that a difference in number of one or two chromosomes might exist between the two clones without cytological detection. On the basis of the cytological findings within this one mutant clone it seems unreasonable to suspect that the hereditary characters of any of the colchicine derived mutant clones depended upon the presence of a polyploid chromosomal complement. At the most it seems that the colchicine treatments may have given rise only to relatively slight numerical aberrations in the normal chromosome complement of Line 1284, which heteroploidy might have been responsible for the mutant characters observed. No precise cytological findings are available to support even this possible interpretation.

DISCUSSION

Of 1748 parthenogenetically produced (diploid) animals hatched from colchicine treated eggs in this study, 60 were decidedly abnormal in one or several respects. Thirty-three of these produced no eggs nor even showed at any time any gross sign of ovarian activity. Twenty others produced eggs (eggs appeared in the brood chamber) none of which hatched. The numbers of eggs produced by animals in this group was quite variable—a few eggs to a nearly normal number. In a third group were seven animals which were stem mothers of mutant clones. Each of these seven produced a number of young and the clones thus obtained were studied for from one to over 60 parthenogenetic generations.

Of 469 control animals studied in parallel with animals from treated eggs none showed abnormalities which were hereditary in any sense. However, out of thousands of animals of Line 1284 studied in other experiments, a very few individuals that produced no eggs have been noted. In one case a spontaneous mutation was detected in Line 1284; this is described in a foregoing paragraph.

The authors consider it unlikely that any of the 60 abnormal individuals reported in this study were tetraploids or polyploids of any sort. This seems the only tenable conclusion, at least for Line 123, in view of the results of the cytological study of this clone.

It is obvious that treatment of eggs with colchicine resulted in a marked increase in the numbers of sterile individuals (producing no eggs) obtained. The very failure of these individuals to produce eggs makes any genetical interpretation of this result highly theoretical. No explanation will be offered here.

The 20 cases of animals hatched from treated eggs which themselves produced eggs incapable of development are probably not to be interpreted as being due to the effects of a direct somatic poisoning. We can scarcely conceive that a sufficient amount of colchicine could have been carried through the development of an individual to so poison eggs produced by that individual as to prevent development of these latter eggs. Apparently in these 20 cases a truly hereditary inability of the eggs to develop was effected by means of the colchicine treatments.

It is paradoxical that these animals themselves should have been able to develop from the treated eggs inasmuch as they apparently bore genetic factors inhibiting development of their own eggs. Most of these individuals, strangely enough, were fairly vigorous as regards their own growth and their viability. These 20 individuals were hatched from eggs which had undergone maturation within the ovaries of normal mothers before the colchicine treatment was applied, or in the earlier series, presumably before the treatments had become effective. The colchicine treatments, for the most part, were not imposed upon the eggs until they had entered the brood chamber at which time the one non-reductional maturation division is well advanced or completed (KÜHN 1908, ALLEN and BANTA 1929). We are inclined to believe that the genetic factors involved in these cases were of such nature as to exert their influence during some ovarian stage of egg development (in the ovaries of mothers which themselves came from treated eggs). Thus eggs produced by these mothers may have failed to develop because of genetic factors operating in a lethal manner at an early stage of their ovarian development or at maturation. This suggestion at least provides us with a conceptual solution of the paradox. By extension of this idea it seems possible to explain the fact that the stem mother of each mutant clone was vegetatively superior to her offspring and likewise superior to all subsequent generations of the derived clone.

For the seven mutant clones discussed in foregoing paragraphs the most reasonable interpretation again appears to be genetic. The term genetic is here used in its broadest sense to include the possibility of chromosome changes of greater extent than single gene mutations. The evidence derived from experiments involving sexual reproduction in Lines 1284 (the normal clone) and 123 (mutant clone derived from Line 1284 by colchicine treatment) serve as one line of evidence that the hereditary change involved in the case of Line 123 was nuclear rather than cytoplasmic. Sexual eggs produced when Line 123 was inbred failed to hatch while a few of the sexual eggs produced by Line 123 females mated with Line 1284 males hatched; and eggs produced by Line 1284 females mated with Line 123 males also hatched in a few cases. It thus appears that a nuclear contribution of Line 1284 was necessary if the egg was to develop. The egg cyto-

plasm of Line 123 did not appear to affect hatchability of the sexual egg when such eggs had been fertilized by Line 1284 males. In the case of Line 123 at least it seems reasonable to conclude that, whatever the nature of the mechanism underlying the characteristics of the mutant clone, it was nuclear rather than cytoplasmic—gene mutations or chromosomal effects, possibly heteroploidy.

In the three distinct cases of reversion of mutant clones to normal we can only suppose that, in some manner, the genetic constitution of the normal parent clone, Line 1284, was restored in the mutant clone.

While in this study a considerable number of colchicine-affected offspring were obtained, some of which were clearly mutants, we find basis for only tentative interpretation. In our opinion the results obtained were based upon gene mutations induced by colchicine treatments or upon chromosomal aberrations not likely to be detected by cytological methods in view of the technical difficulties encountered in such studies. Too little is known genetically and cytologically about this material to provide a basis for final interpretation of the results.

SUMMARY

Parthenogenetic eggs of *Daphnia longispina* were treated with dilute solutions of colchicine at a late stage of ovarian development or during first cleavage stages in the brood chambers of the mothers. Concentrations used ranged from 1/10,000 to 1/10,000,000 parts of colchicine by weight. No atypical individuals occurred among the 469 controls while 60 unusual cases appeared among the 1748 from treated eggs. Of these 60 unusual females 33 showed no ovarian activity, 20 produced parthenogenetic eggs which failed to hatch, and seven were decidedly subnormal reproductively but gave rise to mutant clones which are described.

In three of these mutant clones sudden and complete reversion to normal occurred after a number of generations in the mutant condition. One case of spontaneous mutation occurred in the normal parent stock (Line 1284).

Cytological studies and sexual breeding experiments with one mutant clone in comparison with the normal clone failed to reveal the nature of the genetic changes involved in these mutations. The tentative conclusion that gene mutations or chromosomal aberrations are responsible for the hereditary changes is reached. No evidence for polyploidy is brought forth.

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FURTHER STUDIES OF THE INTERRELATIONSHIPS OF CELLULAR CHARACTERS IN COLUMBIDAE[1]

M. R. IRWIN AND L. J. COLE[2]
University of Wisconsin, Madison, Wisconsin

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EVIDENCE of the separation into distinct parts of the antigenic complex of the erythrocytes of Pearlneck (*Streptopelia chinensis*) not shared with Ring dove, has been given in a previous report (IRWIN 1939). This separation was observed following backcrosses to Ring dove (*St. risoria*) of their species hybrid and selected backcross hybrids. The antigenic complex of Pearlneck has been divided by this method into at least ten characters which have behaved as units, although it is not known with certainty whether one or more genes are acting singly or together in the production of each of these so-called unit-components. This paper presents the results of tests on the relationship of these specific components of Pearlneck with a third species, the Senegal or Palm dove (*St. senegalensis*[3]), and, also, other interrelationships between these three species.

MATERIALS AND METHODS

The details of the immunological methods used to demonstrate (a) the similarities and differences between species in their respective antigenic patterns, and (b) the separation of the different specific Pearlneck components in the cells of backcross hybrids, have already been described (IRWIN and COLE 1936, 1937). Essentially the same methods have been utilized throughout this experiment, for the different absorptions and for the subsequent agglutinations of the various cells with the numerous reagents.

Hybrids have been obtained between Pearlneck and Senegal, and these in turn have produced offspring when mated to Senegal. Although the female hybrids of the cross of Pearlneck and Ring dove have never produced a living squab, the offspring of backcrosses to Senegal of the hybrid between Pearlneck and Senegal were all obtained from two hybrid females. This suggests a closer relationship between Pearlneck and Senegal than between Pearlneck and Ring dove.

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[2] Much valuable technical assistance in these studies has been given by former and present Research Assistants: WARREN G. BLACK, JOHN R. DICK, DRS. ALFRED GOLDEN and GEORGE W. WOOLLEY.

[3] Stigmatopelia of authors; we follow PETERS (1937).

As in previous reports, the senior author has performed the immunological tests, and the junior author has supervised the matings of the birds.

ANALYSIS OF THE SHARING BY SENEGAL OF THE UNIT COMPONENTS SPECIFIC TO PEARLNECK (NOT IN RING DOVE)

Differences in the antigenic components of the Pearlneck and Ring dove are easily demonstrated following the absorptions of the antiserum (produced in the rabbit) for either species by the cells of the other (IRWIN and COLE 1936). It is of considerable interest, then, to determine whether the specific pattern of either of these species, as contrasted with the other, may be shared in part or *in toto* with still other species. Since probably the major part of the specific Pearlneck complex (not in Ring dove) has been separated into its various components, following successive backcrosses to Ring dove, these cellular characters have a very pertinent use as "testers" to assay their respective presence or absence in other species. A parallel study has been reported (IRWIN 1938) on the presence or absence in both Pearlneck and Ring dove of specific components of *Columba guinea* as contrasted with *C. livia*. Although a separation has been observed of the specific constituents of Ring dove, not in Pearlneck, (IRWIN 1939), these have not been obtained in unit form and a comparable analysis for them is not possible.

The results of various tests to determine the sharing or non-sharing by Senegal of the various constituent parts of the specific Pearlneck components (not in Ring dove), are given in table 1. These different component parts, specific to Pearlneck as compared with Ring dove, are called d-1, d-2, . . . d-11, the letter "d" indicating dove (IRWIN 1939).

From the reactions given in table 1, it will be seen that when anti-Pearlneck serum was absorbed by Ring dove cells, it became a reagent which would agglutinate not only the cells of Pearlneck and the F_1 (Pearlneck$\times$Ring dove) but also those of Senegal to practically the same end-dilution. From this result it would be anticipated that probably several of the specific characters of Pearlneck are present also in Senegal, since the reaction with Senegal cells is presumably by virtue of antigens other than those which either species shares with Ring dove.

Confirmatory evidence on this point is gained by observing the interaction of the cells of Pearlneck with anti-Senegal serum absorbed by Ring dove cells. In this reagent, Pearlneck cells were agglutinated at practically the same end-dilution as were Senegal cells, paralleling the results described above when the Pearlneck reagent was used with Senegal cells. These results show that Pearlneck and Senegal have in common one or more antigens, either identical or related, that are not shared by either with Ring dove. It is not surprising, then, to find that this reagent for the

TABLE I

Agglutination interactions, with various absorbed sera, of the cells of Pearlneck, Ring dove, Senegal and of cells carrying one of the species-specific characters of Pearlneck.

ANTISERUM	ABSORBED BY CELLS OF	CELLS: SPECIES				CELLS: UNIT-PEARLNECK SUBSTANCES									
		PEARL-NECK	RING DOVE	SEN-EGAL	$F_1 \frac{P.N.}{R.D.}$	d-1	d-2	d-3	d-4	d-5	d-6	d-7*	d-4 d-8	d-9	d-11
Pearlneck	Ring dove	8, 9	0	7, 8	7, 8	3, 4	3, 4	0, 1, 2	3, 4	5, 6	3, 4	3, 4	3, 4	2, 3, 4	7, 8
Senegal	Ring dove	7	0	8, 9	7, 8	3, 4	3, 4	3, 4	3, 4	5, 6	2, 3	2, 3	2, 3	1, 2, 3	3, 4
Pearlneck	Ring dove and Senegal	7, 8	0	0	7	0	0	0, ±	0	0	++	0, ±	0	0	7, 8
Pearlneck	Senegal	7, 8	0	0	7, 8										
Senegal	Pearlneck	0	3, 4	8											
Ring dove	Pearlneck	0	7, 8	2, 4											
Ring dove	Senegal	2, 4	8	0											

The digits represent the highest dilution of antiserum at which agglutination of the cells was visible. Thus, if the original dilution was one part serum in 45, 1 = 45, 2 = 90, 3 = 180. . . 8 = 5760, the dilutions always doubling. Symbols: C = complete agglutination; ++ = strong agglutination; + = definite agglutination; ± = weak agglutination, and 0 = no agglutination; at the first dilution of antiserum. This was either 1:45 or 1:90.

As in previous reports, to 0.1 cc of the different reagents in successive dilutions was added one drop of a 2 percent saline suspension of the respective cells. Readings were usually made after standing two hours at room temperature or at 31° C.

* According to present tests, at least two substances are represented in these cells.

Senegal specific antigens (not in Ring dove), produced agglutination with nearly all of the cells carrying, respectively, one of the presumed unit-characters of Pearlneck. The same titer of serum was noted for the majority of these different characters, irrespective of whether the Pearlneck or Senegal reagent was used. In fact, the d-3 character was more strongly agglutinated in the Senegal reagent than in most of the Pearlneck reagents that have been available. It would be impossible, by comparing the titers obtained in using these two reagents, to differentiate the agglutination of substances d-1, d-2, d-4, d-5, d-7, d-4.d-8 and d-9, and not surely that of d-6. The d-11 component, however, reacted very differently in these two reagents. The end-dilution at which a reaction occurred for d-11 with the Pearlneck reagent was practically the same as that for either the Pearlneck or F_1 cells (7, 8), while with the Senegal test fluid the titer for this Pearlneck character was no greater than that usually obtained for several other Pearlneck characters (3, 4), as d-1, d-2, etc.

On the basis of these results, we may conclude that Senegal shares with Pearlneck either all or at least a major part of the different cellular characters specific to Pearlneck (contrasted with Ring dove). A more adequate test of the identity or similarity of these characters in Pearlneck and Senegal is possible following an exhaustion of Pearlneck antiserum by the cells of both Ring dove and Senegal. If with this reagent there was no agglutination of cells carrying the d-1 substance, for example, it would be assumed that an antigen indistinguishable from d-1, and therefore presumably identical to it, was present in Senegal cells. But if the reagent agglutinated the cells, as d-6, only a part of d-6, or a substance related to d-6, can be present in Senegal cells. As shown in the table, the titer of this doubly absorbed reagent, for Pearlneck cells and for the d-6 and d-11 components, is the same as that of the test fluid made by the absorption with Ring dove cells alone. But the further absorption by Senegal cells completely removed the agglutinins for the following substances: d-1, d-2, d-4, d-5, d-4.d-8 and d-9, and possibly d-7. (Although d-7 is undoubtedly composed of two antigens (IRWIN 1939) in these tests the cells of a single individual were used and will be considered as but one). Conflicting results have been obtained in tests involving cells carrying d-3, although it is probable that a substance, indistinguishable from d-3 of Pearlneck, is present in Senegal.

We may conclude from these reactions that the following specific characters of Pearlneck (as contrasted with Ring dove) are shared *in toto* by Senegal: d-1, d-2, d-4, d-5, d-4.d-8 and d-9. All, or nearly all, of substances d-3 and d-7 are likewise probably common to Senegal.

Definitely, however, there are no substances in Senegal which are identical with d-6 and d-11 of Pearlneck. That there are cellular characters in Senegal at least related to d-6 and d-11 is evident, since Senegal anti-

serum, when absorbed by Ring dove cells, will react with cells carrying either of these two antigens.

What reasonable explanation, then, can be given of the relationship between the d-6 and d-11 components in Pearlneck and Senegal? If it were known that each of these cellular constituents of Pearlneck was due to the action of a single gene, we would conclude that a gene in Senegal produced a similar but not identical chemical effect. On the other hand, if two or more genes on respective chromosomes produce these two characters in these backcross progeny, another explanation needs to be invoked. Assuming that two or more genes are concerned in either d-6 or d-11, then one or more genes in Senegal may have an effect either identical with or similar to only a part of each of these substances of Pearlneck as they now can be recognized. Parallel relationships have previously been described (IRWIN 1938) between certain unit-characters specific to *C. guinea* (not in *C. livia*) and related components of Pearlneck. Also, the cellular components (A and B, M and N) of humans, each admittedly caused by a *single* gene, appear either as indistinguishable or only as related characters in some of the species of the higher apes and lower monkeys (LANDSTEINER and MILLER 1925, LANDSTEINER and WIENER 1937, WIENER 1938).

SEGREGATION OF SPECIFIC PEARLNECK CHARACTERS FOLLOWING BACKCROSSES TO SENEGAL

According to the results presented above, Pearlneck differs from Senegal chiefly, if not entirely, in that Senegal does not contain more than a part of the (Pearlneck) cellular characters d-6 and d-11. If this be true, the cells of the hybrids between Pearlneck and Senegal should possess these two characters, and the offspring of backcrosses of the F_1 to Senegal would be expected to show a segregation into the four kinds; namely, those whose corpuscles contain (1) both d-6 and d-11, (2) d-6 alone, (3) d-11 alone and (4) neither of the two. (Naturally, this analysis can include only the known constituents of Pearlneck, by which this species differs from Ring dove. Those characters, if any, which are not recognized at present may or may not be shared with Senegal, and constitute an unknown quantity in this analysis).

Two different backcross progenies have been obtained in matings of two hybrid females to Senegal. For each of these, the family is designated by the number, 820 and E11, and the individuals by letters, as 820H, E11B, etc. One of these backcross hybrids (820L) produced offspring (family D967) in mating to a Senegal. The interactions of the cells of some of these backcross birds with various reagents are given in table 2. The majority of these interactions have been made twice, at different times. Owing to the small size of the backcross birds, only a limited amount of blood could be

obtained at any bleeding, thus limiting the volume of the individual reagents.

From these interactions of table 2, it will be seen that the cells of 11 birds of the two backcross families (D820 and E11) gave definite agglutination, and four (D820A and V_2, E11N and D_2) gave only faint traces of agglutination, when tested with anti-Pearlneck serum absorbed by the cells of both Ring dove and Senegal (see first column of data). Following further absorptions by the cells of individual backcross hybrids, the reactions of the respective individual reagents with different cells, and particularly with the "tester cells" for substances d-6 and d-11, allow an analysis to be made of the antigens present in the cells of the different birds. Thus, if by absorption the cells of an individual bird removed the agglutinins for either or both of these specific Pearlneck components, we conclude that the particular antigen was present in the absorbing cells.

For example, when the cells of the F_1 were used in this manner, no agglutinins remained in the absorbed fluid for any of the cells of the birds tested, except for those of Pearlneck. From this result we may say that the species hybrid in this cross probably does not possess quite all of the antigens of the Pearlneck parent, but that it does contain both the d-6 and d-11 antigens, as proposed above. The cells of three birds (D820V, E11P and U) seemingly were identical in antigenic composition with those of the F_1, as shown by the reciprocal interactions (where made) of their respective cells and reagents. The cells of these three birds should then have contained both d-6 and d-11, and the results following their use in respective absorptions show that this was the case.

By the same process of analysis, it is seen from the reactions given in table 2 that the cells of 820H contained substance d-6 but not d-11, while d-11 but not d-6 was found in the cells of 820L, Z and E11B. Similar tests with the cells and the reagents for E11J, K, V and Z established the presence of d-11 in these cells and their antigenic homology with 820L and Z; the details of these tests are not given in the table.

It is probable that the faint reactions noted for the cells of 820A, V_2, E11N and D_2 were due to one or more Pearlneck substances other than d-6 and d-11, since the reagents for the cells of these four different individuals readily agglutinated both tester substances, d-6 and d-11. Conversely, the reagents for d-6 and d-11 produced faint but definite agglutination with each of these different cells, thus practically eliminating the possibility that there had been a fractionation of either of these characters and that a part of one or the other was responsible for the faint reactions shown for them (first column of data in table 2).

Furthermore, since the reagent for d-6 agglutinated the cells of 820H, which contained d-6, and that for d-11 also agglutinated the cells contain-

TABLE 2

Interactions of the cells of "quarter-Pearlnecks" (from a cross of Pearlneck×Senegal) with various reagents.

CELLS	TITERS FOLLOWING ABSORPTION OF PEARLNECK ANTISERUM BY RING DOVE AND SENEGAL CELLS	TITERS OF PEARLNECK ANTISERUM, FIRST ABSORBED BY RING DOVE AND SENEGAL CELLS, THEN BY THE CELLS OF EACH OF THE FOLLOWING BIRDS:													
		F_1	820A	820H	820L	820V	820Z	$820V_2$	E11B	E11N	E11P	E11U	$E11D_2$	d-6	d-11
Pearlneck	7, 8	±	7	7	3, 4	±, +	4	7	3, 4	7	+, 2		7	7, 8	2, 4
Ring dove	o	o	o	o	o	o	o	o	o	o		o	o	o	o
Senegal	o	o	o	o	o	o	o	o	o	o	o	o	o	o	o
F_1(P.N./Sen.)	7	o	++	++	2	o	2	++			o				
820A	o, +	o	o	o	o	o	o	o			o				
820H	3, 4	o	++	o	±, ++	o	++	++	+, ++	±	o	o	±	+	
820L	7	o	C	C	o	o	o	C	o		o	o			±
820V	7	o	C	C	++	o	++	C							
820Z	7	o	++	C	o	o	o	C							
$820V_2$	o, +	o	o	o	o	o	o	o							
E11B	7, 8								o						+
E11J	7				o				o			o			+
E11K	7														
E11N	±, 2			o						o	o		o	±	
E11P	6, 7					o					o				
E11U	7											o			
E11V	7														+
E11Z	7														+
$E11D_2$	2			o	o				o	o		o	o	+	
d-6	2, 3	o	++	o	+, ++	o	++	+	+, ++	++	o	o	+	o	++
d-11	7, 8	o	C	C	o	o	o	C	o	C	o	o		++, C	o

For explanation of digits and symbols, see table 1.

ing d-11 but not d-6, as 820L, Z, E11B, etc., we must ascribe to these cells of the different birds tested another property specific to Pearlneck than the two recognized (d-6 and d-11). As described above, those respective results differ from the reciprocal tests, since the reagents for each of the backcross individuals whose cells contained d-11 failed to agglutinate the "tester" cells for d-11. We are unable at present to say whether or not this additional substance is a part of one of the known unit components of Pearlneck (as contrasted with Ring dove).

Exclusive of this unknown character, which is temporarily designated as the "X" constituent, the birds of the backcross offspring may be grouped in the four classes as proposed above:

	Cells containing			
	d-6.d-11	d-6	d-11	neither d-6 nor d-11
Number of birds	3	1	7	4

These are reasonably close to the equality in numbers expected in each of the four classes, as proposed above. One bird (820L), whose cells contained the d-11 and not the d-6 character, produced offspring of the second backcross generation, in mating to Senegal. The expectation would be that the progeny would consist of two classes with approximate equality: those with and those without the d-11 component. Actually, of nine birds raised, three possessed this character and six did not. And since the cells of 820L contained the "X" substance as well as d-11, four kinds of cells would be expected in its backcross progeny. The actual findings follow:

	Cells containing			
	d-11."X"	d-11	"X"	neither d-11 nor "X"
Number of birds	2 (or 1)	1 (or 2)	3 (or 4)	3 (or 2)

Because of the faint reaction usually typical of the "X" substance, there was uncertainty in classifying its presence or absence in the cells of four birds, and the number in these four groups is, therefore, more or less tentative.

INTERRELATIONSHIPS OF PEARLNECK, RING DOVE AND SENEGAL

Evidence has been presented above that Pearlneck and Senegal share many genetic characters (cellular) which are not found in Ring dove. If Senegal also possesses either all or the majority of the substances common to Pearlneck and Ring dove, there would be but little question that Pearlneck and Senegal were more closely related than Pearlneck and Ring dove.

This relationship can be tested in agglutinations following the respective absorptions by Senegal cells of anti-Pearlneck and anti-Ring dove sera. The results from these tests are at variance, for although the various Pearlneck antisera, absorbed by Senegal cells, did not agglutinate Ring dove cells, Pearlneck cells are agglutinated by Ring dove antiserum when absorbed by Senegal cells. These Pearlneck antisera were seemingly alike in that they each lacked the one or more agglutinins for the part of the Pearlneck antigenic complex common to Ring dove but not common to Senegal.

Utilizing the interactions with Senegal and Ring dove cells following the absorption by Pearlneck corpuscles of antiserum for each of these two species, as given in table 1, we note that Senegal and Ring dove have in common probably a minor part of their respective antigenic complexes to the exclusion of Pearlneck.

Thus in Pearlneck, in comparison with Senegal and Ring dove, a part of the antigens are found only within the species; the remainder are shared, in varying proportions, with Ring dove only (probably), with Senegal only and with both Ring dove and Senegal. A similar complex exists for both Ring dove and Senegal, since each contains antigens specific to itself, and shares other antigens with the other two species, singly and together.

If more related species were used in these comparisons, would it be found that any single species possessed antigens peculiar to itself alone; that is, not shared in any other species? Or might the antigens, as of Pearlneck, be simply a composite sample of certain of those found in several other species? Pearlneck then would differ from these other species only in being a different combination of antigens (genes), all of which were present in a group of related species. Data pertinent to this question will be considered elsewhere.

DISCUSSION

The experiments described in this paper represent essentially the agreement between probable relationships between two species, as deduced simply from immunological tests, and the results obtained when this relationship was tested genetically. Having given "tester" cellular characters, by which Pearlneck differs from Ring dove, and having found that a part of the specific antigenic pattern of Pearlneck is shared with a third species, Senegal, the next step was to determine which of each of the ten specific characters of Pearlneck was contained wholly or in part in the cells of Senegal. It was found that all or nearly all of eight of the ten antigens specific to Pearlneck, and only a part of the other two substances (d-6 and d-11) were present in Senegal cells. The offspring of hybrids between Pearlneck and Senegal, when backcrossed to Senegal, should then be of the four different types possible following the segregation of these two characters.

The actual findings on the cells of the offspring were in accordance with the expectation. These results give added confidence that similar comparisons between species are an index of the genetic relationships between them, even though hybridization may not be possible between the species being compared.

These findings show very clearly that the characters (therefore the causative genes) by which one species (Pearlneck) differs from another (Ring dove) may be shared at least in part by still a third species (Senegal). If the cellular characters specific to Pearlneck, as contrasted with both Ring dove and Senegal, are shared with one or more other species, our concept of the genetic make-up of this species would be that it was simply a peculiar combination of genetic characters which were present in several different species. The other possibility would be that some species, perhaps all, possess genetic characters particular only to themselves. Experiments designed to provide an answer to this problem will be reported in other papers.

Because of the relationship shown to exist between the Pearlneck and Senegal species, and of both of these to Ring dove, another possibility in the analysis of the genetic relationships between species is indicated. If hybrids can be obtained between Senegal and Ring dove, in backcrosses to Ring dove there should eventually be produced in unit form the cellular characters specific to Senegal. Many of these should presumably be indistinguishable from, and, therefore, presumably identical with, the unit characters of Pearlneck. The questions eventually to be answered are, then, whether each unit substance of Pearlneck is also a unit-substance of Senegal, or whether any two or more, or parts of the respective Pearlneck characters behave as units in Senegal. Considerable progress has already been made in the production of the backcross progeny of this cross. Future reports will deal with the segregation of specific Senegal characters observed among them.

SUMMARY

All or nearly all of eight, and only a part of the remaining two of the ten specific cellular characters of Pearlneck, not in Ring dove, are indistinguishable from and presumably are identical with antigens present in the cells of Senegal. The expected segregation of the Pearlneck characters, not present *in toto* in Senegal, was observed in the offspring of backcrosses to Senegal of the species hybrid (Pearlneck × Senegal). There appeared to be still a third character, in addition to these two recognizable constituents, which differentiated Pearlneck from Senegal.

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GENETIC DATA ON DROSOPHILA AFFINIS, WITH A DISCUSSION OF THE RELATIONSHIPS IN THE SUBGENUS SOPHOPHORA

A. H. STURTEVANT
California Institute of Technology, Pasadena, California

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INTRODUCTION

DROSOPHILA affinis Sturtevant is a member of the subgenus Sophophora (STURTEVANT 1939), closely related to *D. athabasca, azteca, algonquin, narragansett,* and *seminole* (STURTEVANT and DOBZHANSKY 1936b). Cytologically, all the members of this group (*seminole* has not been examined) are aberrant in the subgenus in that they have—in addition to a large V-shaped X chromosome and a small dot-like autosome—three two-armed autosomes (V-shaped or J-shaped—see figure 1). The short arm of one of the J's appears to be wholly heterochromatic, but there remain five autosomal euchromatic arms in addition to the dot—that is, including X and the dot, a total of eight. This was shown by MILLER 1939, for *algonquin;* for *azteca* and *athabasca* this conclusion rests on unpublished information communicated by PROF. T. DOBZHANSKY and MR. E. NOVITSKI. In most other members of the subgenus (*melanogaster, pseudoobscura, subobscura,* etc.), the total number of euchromatic arms is six. As indicated later in this paper, the European *obscura* probably agrees with the *affinis* group, *ananassae* has an extra X-arm, and in *willistoni, earlei,* and their relatives the dot has disappeared. The study of mutant genes in *affinis* was undertaken in order to throw some light on the relation between the distribution of genes in the *affinis* configuration and that in the other members of the subgenus.

MATERIAL

D. affinis occurs in the eastern United States, from central and southern Texas, to southeastern Kansas, the southern shores of the Great Lakes, Massachusetts, and the central portion of the Florida peninsula. Along its northern boundary *affinis* is gradually replaced by *athabasca* (subspecies *mahican*), along its western boundary (at least in Texas) by the ecologically similar *pseudoobscura*—these two species themselves showing a similar replacement along a zone from British Columbia to Colorado (STURTEVANT and DOBZHANSKY 1936b). *D. algonquin* is found along the whole western and northern replacement zone for *affinis*, being present, so far as known, only in areas where *affinis* occurs along with either *pseudoobscura* or *athabasca*. The *affinis-athabasca* replacement zone is also occupied by

D. narragansett (Woods Hole, Massachusetts; Darien, Connecticut; Ithaca, New York; Wooster, Ohio). *D. seminole* is known only from southern Alabama, deep within the range of *affinis*. It will not be surprising if the ranges of *D. azteca* and *affinis* are found to overlap on one side or the other of the Rio Grande. Thus this species is in contact, or nearly so, with all the other known members of its group, a relation scarcely possible for any of the others, since *seminole* at least seems very unlikely to occur with any of them.

Two subspecies of *D. affinis* have been recognized (STURTEVANT and DOBZHANSKY 1936b), the typical one being lighter in color and more southern in distribution than the subspecies *iroquois*. Study of a large series of living strains from many parts of the range shows that, in fact, southern strains average lighter in color than northern ones. However, both extremes of color may be found throughout the range, and there is no trace of cross-sterility or of any associated differences between the types. The few attempts that have been made to study the inheritance of the difference have not been encouraging. There is so great an age and environmental effect on the character as to make it very difficult to work with. For present purposes it has seemed simplest to ignore the subspecific distinction entirely.

The wild strains used in the present study have come from many regions, and many of them have been collected by friends. I am especially indebted to PROF. J. T. PATTERSON for material from Texas and Louisiana, to PROF. W. P. SPENCER for material from Kansas, Missouri, Tennessee, Ohio, and western New York, and to DR. D. F. POULSON for strains from Maryland and Connecticut. My own collections have been from Massachusetts and Alabama. The wild females have been brought in, and F_2 generations reared from each one separately. In this way a number of mutant types have been isolated; others have appeared in old strains and in experiments concerning the linkage relations of previously found types.

There follows a catalogue of the mutant types studied, arranged by chromosomes, together with a discussion of the possible parallelism of each to types known in other species.

MUTANT TYPES

Left limb of the X chromosome

bobbed. Found in a wild strain originally from Chautauqua, New York. Bristles small, abdominal tergites with rather few bristles and with etched margins. Resembles the bobbed of *melanogaster*, *simulans*, *pseudoobscura*, etc. (see LÜERS 1937). So far as tested, the Y chromosome is without effect on the bobbed character, there being no noticeable sexual dimorphism for it. Y chromosomes from Massachusetts, New York, Tennessee, and Kansas have been studied. Bobbed females have been sterile so far as tested.

cut. Found in a wild strain originally from Woods Hole, Massachusetts. Wings with nicks in margin; often extreme, sometimes overlapping wild type, especially in females. Often the humeri are more separate from the mesonotum than usual, suggesting epaulets. Except in this last respect the character is much like the beaded of *pseudoobscura*, and also resembles cut of *melanogaster*.

pebbled. Found in experiments with a cut vermilion chromosome. Wing surface coarsely granular. Often of low viability and fertility, but is easily kept in one homozygous strain that apparently happens to have modifiers favorable for it.

scute-1. Arose in linkage experiments. Resembles the scutes of *melanogaster*, scutellar of *pseudoobscura*, etc. Both scutellar bristles are usually absent, and the anterior and middle orbitals are often absent. In some cultures a dorsocentral is also missing. The character is variable, and sometimes overlaps wild type, especially in females.

scute-2. Arose in experiments involving scute-1, and was at first confused with it. Resembles scute-1, but is less viable and fertile, and less frequently affects the dorsocentral bristles.

stubby. Found in linkage experiments. Bristles slightly shortened and curved. Perhaps a slight allele of either forked or singed (*melanogaster*, *simulans*, *pseudoobscura*, and other species).

vermilion. Arose in the cut stock. Quite similar to the vermilion of *melanogaster* and other species. MR. C. W. CLANCY tested it for me by the feeding technique of BEADLE and LAW (1938), and reports that it lacks the v^+ substance that is present in wild-type *affinis* (vermilion brown *melanogaster* larvae were used as test material). There can therefore be no doubt of the identity of the locus.

white-1. Arose in the same culture as scute-1, but in the other X of the mother of that culture. Eyes completely white, as in the well-known types of *melanogaster* and other species.

white-2. Arose in a scute-1 scarlet chromosome. Completely white eyes, allelic to white-1.

Right limb of the X chromosome

ascute. Two males were found in a linkage experiment. Resembles ascute and grooveless of *melanogaster* or of *pseudoobscura*.

hairy. Found in a strain from Gatlinburg, Tennessee. There are a few hairs on the dorsal surface of the scutellum. The character is variable; often only one hair is visible, and sometimes the specimens look wild type. In many crosses some of the heterozygous females have one or two of these hairs present. The resemblance to hairy of *melanogaster* would be of doubtful significance were it not in agreement with the other parallels in this arm.

hairy-2. Found in linkage experiments with cut and scarlet. It is indistinguishable from hairy-1, and allelic to it.

scarlet. Arose in a cut vermilion stock, where its presence was only detected as a result of apparent inconsistencies in linkage experiments, since vermilion, scarlet, and vermilion scarlet are indistinguishable in eye color. The resemblance to scarlet of *melanogaster* or *pseudoobscura* is clear.

sex-ratio. Found frequently in wild strains from various localities (Woods Hole, Massachusetts; Coffeyville, Kansas; Gatlinburg, Tennessee —see STURTEVANT and DOBZHANSKY 1936a). This gene is not easy to study in *affinis*, since its expression is variable; males carrying it often give essentially normal sex-ratios, yet descendants carrying the same X may give large families with few or no sons. For this reason statistics concerning the frequency of the gene in wild populations are of little value. Sex-ratio has been found associated with only one of the two sequences referred to below (the inverted one); it remains uncertain whether it is always present in chromosomes having that sequence.

tilt. Found in the original white-1 chromosome. The wings are often curved upwards slightly at their tips, but a more useful index is the presence of extra veins between the second and third longitudinal veins at about the level of the anterior crossvein, the two longitudinals often being fused here into a knot. The character is variable, frequently overlapping wild type, especially in females. Resembles tilt of *melanogaster*.

veinlet. Found in early experiments with white-1, of the same series of cultures as that in which tilt was found. The third, fourth, and fifth longitudinal veins are often incomplete at their distal ends. Overlaps wild type. Resembles short of *pseudoobscura*, or veinlet of *melanogaster*, though it is less extreme than the latter.

Second chromosome

abnormal. Found in a strain from Baltimore, Maryland. Abdominal bands irregular, tergites often failing to meet in the mid-dorsal line. Overlaps wild type.

arc. Found in a wild stock from Woods Hole, Massachusetts. Wings rather short, bowed as in the arc of *melanogaster*, but with no approximation of the crossveins. Overlaps wild type.

bithorax. Found in linkage experiments with chromosome 5. The balancers are wing-like. Overlaps wild type. Resembles some of the alleles of bithorax in *melanogaster* or *pseudoobscura*.

claret. Present in a wild strain from Baton Rouge, Louisiana. Resembles pinkish and pink-like of *affinis*, pink and claret (as well as other eye colors) of *melanogaster*, *simulans*, or *pseudoobscura*.

crossveinless. Found in the stock of pinkish, of pure Woods Hole ancestry. Posterior crossvein broken or absent. Overlaps wild type frequently. Resembles crossveinless-d of *melanogaster;* not like crossveinless of *melanogaster* (X) or of *pseudoobscura* (2).

crumpled-1. Found in F_2 from a wild female from Litchfield, Connecticut. Wings abnormal, distal part often folded back under proximal part; branches of arista bent anteriorly near their bases, so that distal parts are parallel to main axis; bristles blunt, somewhat shortened; fertility greatly reduced. Resembles crumpled of *melanogaster* or *pseudoobscura.*

crumpled-2. Found in linkage experiments. Phenotypically not distinguishable from crumpled-1; allelism and linkage not tested.

Delta. Found as two individuals in a linkage experiment. Dominant. Veins thickened, especially at junctions, acrostichal hairs numerous and in irregular rows, eyes somewhat roughened, tarsi short and thick. Lethal in homozygous condition. Resembles Delta of *melanogaster* or *simulans* and Smoky of *pseudoobscura.*

knot. Found in a strain from Woods Hole. Slight thickening of junctions of veins. Overlaps wild type. Not easily classified.

pinkish. Found in F_2 from a wild female from Woods Hole, Massachusetts. Pink eye color, resembling pink, claret, ruby, garnet, etc. of *melanogaster,* or pink, claret, magenta and ruby, of *pseudoobscura.*

pauciseta. In a wild stock from Gatlinburg, Tennessee. Many of the bristles and hairs are reduced in size or absent, in an irregular pattern. Often so nearly wild type as to be difficult to classify. Resembles pauciseta of *pseudoobscura,* and may also be compared to recessive hairless of *simulans.*

rugose. Found in F_2 from a wild female from Lake of the Ozarks, Missouri. Eyes distinctly rough, of normal size and convexity.

short-wing. In the wild stock from Woods Hole, Massachusetts that contained knot. The two genes were originally associated in the same chromosome. Wings short, blunt, of uneven texture. Variable, and sometimes overlaps wild type.

short-1. Found in linkage experiments. Character similar to veinlet, but somewhat more extreme and less often overlapping wild type.

short-2. Found in F_2 from a wild female from Gatlinburg, Tennessee. Not different phenotypically from short-1, to which it is allelic.

short-tarsi. Found in F_2 from a wild female from Mendham, New Jersey. Legs, especially tarsi, short. Often a strong constriction in the tarsi, leaving the terminal segments attached to the rest of the legs by a thread-like connection. Arista often with a thickened axis.

tiny-bristle. Found in a stock from Wooster, Ohio. Bristles fine, not shortened. No marked effect on development rate.

Third chromosome

cinnabar. Present in a wild strain from Austin, Texas. Bright eye color, not separable from vermilion or scarlet. Feeding experiments made for me by MR. D. D. MILLER show that it has v^+ but not cn^+ substances, that is, that it corresponds to cinnabar of *melanogaster* and orange of *pseudoobscura*.

narrow. Found in linkage experiments. Wings narrow, cross veins approximated; legs rather short; head and mesonotum rather short and broad, sometimes slightly concave in mid-dorsal line. Resembles lanceolate of *melanogaster*, or narrow of *pseudoobscura*.

pink-like. Found in F_2 from a wild female from Woods Hole, Massachusetts. Like pinkish, but slightly more extreme. This, like pinkish, gives, when combined with vermilion, a clear yellow eye-color.

roughish. Found in a strain from Coffeyville, Kansas. Eyes rough, often somewhat wrinkled; various head and thoracic bristles may be absent; variable, sometimes quite extreme but may overlap wild type.

straw. Found in linkage experiments. Yellowish body-color, quite similar to the straw of *melanogaster* or of *simulans*.

Fourth chromosome

antennipedia. Found in F_2 from a wild female from Litchfield, Connecticut. The antennae are more or less leg-like, varying from normal antennae up to an extreme in which a practically complete leg (femur, tibia, basal tarsal segments), bearing a rudimentary arista at its tip, is attached to the rudimentary base of the third antennal segment. The type differs radically from the aristapedia of *melanogaster* or *simulans*, where the normal antenna becomes more or less leg-like—the second antennal segment becoming femur-like, the third tibia-like, and the arista quite tarsus-like. In antennipedia all the normal antennal parts persist, including an arista (which may be short, thick, or rudimentary, but is inserted at the normal position), while the apex of the third segment has some portions of a leg attached to it. Another property of antennipedia is that, in the males, sex-combs are often present on the tarsi of the second and third pairs of legs (more often the second) as well as the first where they normally occur. This applies more particularly to the longer sex-comb of the basal tarsal segment; the single tooth of the comb on the second segment is sometimes present on the second leg, but has not been seen on the third.

jaunty-1. Found in F_2 from a wild female from Gatlinburg, Tennessee. Resembles jaunty of *melanogaster* or of *pseudoobscura*, curled of *melanogaster*, upturned of *pseudoobscura*, etc.

jaunty-2. Found in F_2 from a wild female from Mendham, New Jersey. Like jaunty-1 and allelic to it.

jaunty-3. Found in F_2 from a wild female from Baton Rouge, Louisiana. Like jaunty-1 and allelic to it.

TABLE I

Corresponding mutant types.

affinis	*pseudoobscura*	*melanogaster*
XL	XL	X
bobbed	bobbed	bobbed
cut	beaded	cut
miniature	miniature	miniature
scute	scutellar	scute
vermilion	vermilion	vermilion
white	white	white
yellow	yellow	yellow
XR	XR	3L
ascute	ascute	ascute
hairy	—	hairy
scarlet	scarlet	scarlet
sex-ratio	sex-ratio	—
tilt	tilt	tilt
veinlet	short	veinlet
2	2	3R
bithorax	bithorax	bithorax
claret	pink, claret	pink, claret
crossveinless	—	crossveinless-d
crumpled	crumpled	crumpled
Delta	Smoky	Delta
pinkish	pink, claret	pink, claret
pauciseta	pauciseta	(Hairless ?)
3	3	2R
cinnabar	orange	cinnabar
narrow	narrow	lanceolate
straw	—	straw
4	4	2L
jaunty	jaunty	jaunty
net	tangled	net
truncate	—	dumpy
5	5	4
abdomen rotatum	—	abdomen rotatum
reduced	—	(shaven ?)

net. Present (heterozygous) in a wild male from Ithaca, New York. Extra wing-veins, especially between the distal ends of the second and third veins, and around the posterior crossvein. Resembles net of *melanogaster*, or plexus and tangled of *pseudoobscura*.

truncate. Found in linkage experiments. Wings short, often with the characteristic "truncate" shape, that is, strongly indented at the posterior distal corner, but with intact margin. Overlaps wild type. Resembles some of the dumpy alleles of *melanogaster*

Fifth chromosome

abdomen rotatum. Found in linkage experiments. The abdomen is twisted about its longitudinal axis in a counterclockwise spiral. Both sexes are sterile. Resembles the abdomen rotatum of *melanogaster*.

Fused. Arose in a cross between a strain from Woodstock, Maryland, and one that carried several mutant genes. Dominant, lethal when homozygous. Third and fourth veins strongly convergent at their distal ends; ocelli and ocellar bristles usually absent. Resembles the fused of *melanogaster*, except that that type is recessive and has a peculiar type of lethal action (LYNCH 1919) that is not evident in *affinis*.

reduced. Found in a strain from Woodstock, Maryland. Posterior scutellar bristles (occasionally also the anterior ones, rarely a few other thoracic bristles) very short, thick, and blunt. Suggests the shaven of *melanogaster*, though not a convincing parallel.

CHROMOSOME MAPS

The X chromosome

Two different sequences occur in each arm, the "standard" sequence of XL being regularly associated with the standard of XR, and the inverted sequences being likewise associated. Both sequences have been found in strains from Woods Hole, Tennessee, Missouri, Kansas, and Texas. The association between the two limbs remains unexplained, but can probably be interpreted when salivary gland preparations are studied.

Scute-1, white-2, and hairy-1 arose in the inverted sequence; scute-2, white-1 and hairy-2 in the standard. Ascute, tilt, and scarlet all arose in standard, but were later transferred to the inverted sequence by crossing over. These six genes are therefore known in both sequences; the others only in one or in the other. Maps are shown for each sequence.

The maps are unsatisfactory, since many of the mutant types are unsuited to exact linkage studies. Pebbled, bobbed, scute-2, and ascute all have markedly low viability; cut, both scutes, both hairys, tilt, and veinlet, all overlap wild-type; and white, vermilion, and scarlet interfere with each other's classification.

In females heterozygous for the two sequences there is very little crossing over, not over one percent for the whole known region. Most or all of that which occurs is in the region to the right of hairy. Patroclinous sons have been found rarely from such females, but it is not certain that they

were XO in constitution, so no definite conclusions can be drawn from them.

Perhaps the most unusual relation shown here is that yellow and scute are not close to each other as in most species, but are separated by cut, vermilion, and pebbled.

TABLE 2

Linkage maps of X, second and fourth chromosomes of D. affinis.

STANDARD SEQUENCE		INVERTED SEQUENCE	
stubby	0	miniature	0
white	2	scute	6
yellow	12	white	12
cut	17	hairy	28
vermilion	22	veinlet	40±
pebbled	24	tilt	40±
scute	27	ascute	60
bobbed	40±	scarlet	90
hairy	40±		
veinlet	50±		
tilt	50±		
ascute	60		
scarlet	99		

SECOND CHROMOSOME

pauciseta	0
short-wing	1±
rugose	25
pinkish	33
crumpled	45
short	67±
Delta	68
crossveinless	68±
bithorax	Between 66 and 70
abnormal	73±
tiny-bristle	98
short-tarsi	98±

FOURTH CHROMOSOME

net	0
antennipedia	31
jaunty	67

(Loci marked "±" are uncertain as to order, as well as to numerical value.)

MAPS OF AUTOSOMES

The second chromosome map shown is more satisfactory than that of the X, since there are more useful characters here. No variations in linkage have been found, indicating that the sequence shown is the usual one throughout the range of the species.

The possible combinations of the three third chromosome characters pink-like, narrow, and roughish have all been studied rather extensively, but no double recessive has yet been obtained. Either all three loci are close

together, or the chromosomes concerned have different sequences. The other mutant genes in this chromosome have not yet been adequately tested.

The fourth chromosome map, like that for the second, shows values that have been obtained repeatedly from crosses to a wide variety of strains. It must represent the sequence usual throughout the range of the species. Preliminary studies show that truncate is approximately 34 units from net; it has not yet been placed with respect to the other two loci shown on the map.

The fifth chromosome has been little studied. Reduced is not favorable for study, since it overlaps wild type frequently, and the complete sterility of abdomen-rotatum makes it also unfavorable. The few tests carried out have not yielded any crossovers between either of these and Fused.

PARALLELISM WITH MUTANT TYPES OF OTHER SPECIES

Comparison of the mutant types here described with those known in *melanogaster* or in *pseudoobscura* (see DONALD 1936, STURTEVANT and TAN 1937) leaves little doubt as to the homologies, which are evidently as follows:

affinis	XL	XR	2	3	4	5
pseudoobscura	XL	XR	2	3	4	5
melanogaster	X	3L	3R	2R	2L	4

In the case of the X this relation was to have been expected, since the X of *melanogaster* seems to include material that is in the X of every species of Drosophila yet studied, while the presence of the sex-ratio gene had previously indicated that the XR of *affinis* agrees with that of *pseudoobscura*. The composition of the fifth chromosome is also the expected one. With respect to the three large autosomes there was no satisfactory basis for a prediction of the constitution of *affinis*. The present data are themselves not sufficient to furnish a final solution here, especially since the position of the centromeres on the maps remains unknown, and the number of parallels is still small. It is still possible that the parallel genes in one or two of the *affinis* autosomes are all in one arm, so that the homology given is not certainly applicable to the entire chromosomes. Nevertheless, the absence of any parallels in disagreement with the suggested correspondence is striking, and may be taken as raising a strong presumption that each autosome of *affinis* contains essentially the same material as does one of those of *pseudoobscura*.

The implications of this result will be discussed below.

THE SUBGENUS SOPHOPHORA

The subgenus Sophophora falls naturally into four species groups, as follows:

1. Dark species; long fine ventral receptacle; long spiral testes; one or

two opaque heavily chitinized areas on the fifth abdominal tergite of the female; skipping larvae; no sex combs; filaments of eggs much expanded at apices. Found in tropical America. *D. saltans* and six undescribed species.

2. Yellow species; long fine ventral receptacle; medium long spiral testes; no opaque areas on tergites; larvae do not skip; no sex-combs; egg filaments much expanded at apices. Found in tropical America. *D. willistoni* and *nebulosa*.

3. Yellow species; rather long ventral receptacle; medium long spiral testes; no opaque areas on tergites; larvae do not skip; sex-combs present. Found in Old World tropics (the first three also in America, where they appear to be introduced). *D. melanogaster*, *simulans*, *ananassae*, *takahashii*, *montium*, *auraria*, *bipectinata*, etc. *D. miki*, from Austria, probably also belongs here.

4. Dark species; short ventral receptacle; testes elliptical or somewhat elongate, scarcely spiral; no opaque areas on tergites; larvae do not skip; sex-combs present; preapical bristles on anterior tibiae unusually long. Found in the north temperate zone (*azteca* and *pseudoobscura* reach Guatemala, but only in the mountainous temperate regions). *D. obscura*, *subobscura*, *pseudoobscura*, *miranda*, *affinis*, *algonquin*, *azteca*, *athabasca*, *narragansett*, etc.

At first glance, the relationships between these four groups appear to be those indicated by the sequence given, that is, the second group is intermediate between the first and third, the third between the second and fourth, and the first and fourth most distinct.

The chromosome configurations shown by these species are diverse. *D. willistoni* (group 2) has a V-shaped X, a V-shaped autosome, and a rod-shaped autosome (LANCEFIELD and METZ 1921), as shown in figure 1. *D. saltans* is described as having the *melanogaster* oögonial configuration, which differs only in having a small dot, though the similarity is misleading, since in *melanogaster* the rod, rather than one of the V's, is the X. MR. E. NOVITSKI, in this laboratory, reports that the three *saltans* relatives he has studied have no dot; they may be supposed to have the same complex as *willistoni*. *D. earlei* (from Cuba) was described by METZ (1916b) as having the *willistoni* oögonial configuration; the species is evidently related to the *saltans* group, though it cannot be determined whether it should be placed with the first or the second group here defined (the larvae were not observed to skip, and in the available pinned material it is not possible to be certain whether an opaque area is present on the abdominal tergite). *D. nebulosa* is also listed as having the *melanogaster* oögonial configuration; but examination of the published figures (METZ 1916a) shows that no dots were found. It seems probable that the *willistoni* complex is characteristic of both the first and the second species groups.

Both the third and the fourth groups have a variety of configurations. The *melanogaster* type is found in *simulans*, and is recorded by KIKKAWA and PENG (1938) for *auraria*, *lutea*, *rufa*, and *takahashii*, with *ficusphila* and *suzukii* probably the same but without a positive identification of the X. All these forms apparently belong to the third group of species. (In the cases of *lutea*, *rufa*, *ficusphila*, and *suzukii* this reference is based on the published descriptions, since I have seen no material.) Also in this group

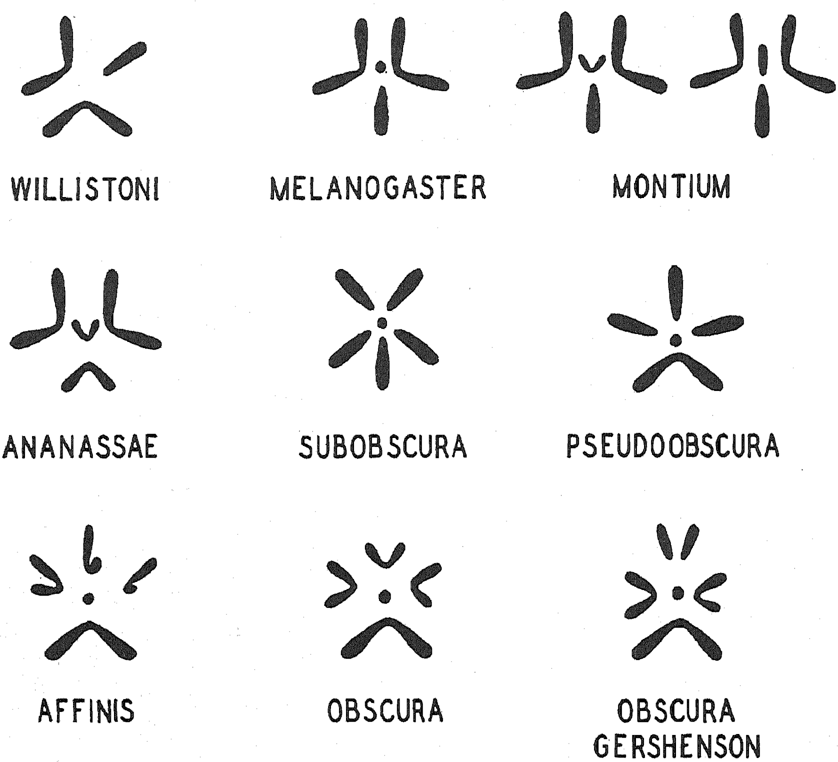


FIGURE 1.—Diagrammatic representation of the chromosome configurations known in the subgenus Sophophora. Haploid groups are shown. In every case the lowermost chromosome is the X; the Y, which is variable even within some of the species, is not shown.

are *montium* and *ananassae*, with the configurations shown in figure 1. *Bipectinata* resembles *ananassae* (KIKKAWA and PENG 1938), though the X has not been identified. KIKKAWA'S (1937, 1938) studies show that *ananassae* has the *melanogaster* arrangement with two modifications: the basal portion of X, including much of its heterochromatin, the nucleolus, and the gene bobbed, has become attached to the dot; and the remaining portion of X has acquired a median centromere. The configurations found in *montium* presumably represent the first of these changes (or one similar

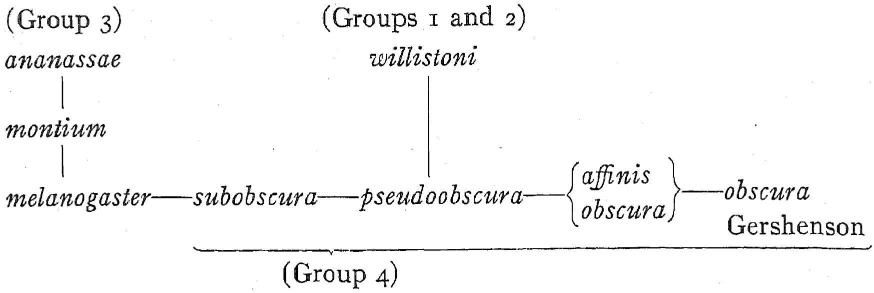
to it—there is no evidence to show how much of X has been transferred to the dot), without the second one. The third group of species is thus characterized by the *melanogaster* configuration or relatively slight modifications of it.

The fourth group of species includes the last five types shown in the figure. Of these, the *affinis* and *obscura* arrangements (found also in *algonquin*, *azteca*, and *athabasca*) are essentially the same, differing only in the relative lengths of the arms of the autosomes. The "*obscura* Gershenson" configuration differs in that one of the autosomes is represented by two rods (see FROLOWA and ASTAUROFF 1929 for the two *obscura* types). *Pseudoobscura* and *miranda* differ from the *affinis* type in that the three J-shaped autosomes have all become rods, and that at least two of them appear to be shorter than in *affinis* or *obscura*. Finally, *subobscura* differs from *pseudoobscura* in having one arm of the X now present as an additional autosome.

The comparison of mutant genes in *melanogaster*, *simulans*, *willistoni*, *pseudoobscura*, and *miranda* shows that in these species the separate arms maintain their identities (see discussion by STURTEVANT and TAN 1937). The X of *melanogaster* and *simulans* remains as one arm of X in the other three species, and the other arm of X in these three species is the equivalent of 3L of *melanogaster* or *simulans*. The present account confirms the suggestion of STURTEVANT and DOBZHANSKY (1936) that the X of *affinis* has the same composition as that of *pseudoobscura*. The work of KIKKAWA (1937, 1938) shows that the X of *ananassae* is the same as that of *melanogaster*, except that the bobbed end has been removed and attached to the dot, while the centromere of the remaining portion has become median. The composition of the X is thus fairly clear throughout the subgenus.

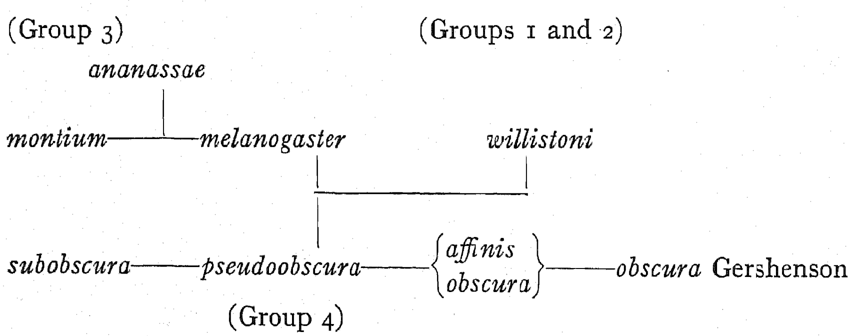
With respect to the autosomes there are two chief problems: (1) where is the dot material in the *saltans* and *willistoni* configuration; (2) what is the content of the J-shaped autosomes of *affinis* and *obscura?* The first question cannot be answered from the available evidence; the second is answered in part by the data presented in this paper. It is probable that each autosome of *affinis* corresponds largely or entirely to one autosome of *pseudoobscura* or to one autosomal arm of *melanogaster*. The changes involved have, then, apparently been merely in the position of the centromeres and associated heterochromatin. This interpretation must be accepted with caution, however, as pointed out above.

The historical interpretation suggested by these configurations is indicated in the accompanying diagram. It should be emphasized that the names in the diagram refer to chromosome configurations as shown in figure 1, not to the species themselves.



Analysis of the characters of the animals by the methods suggested previously (STURTEVANT 1939) is in agreement with this arrangement except that groups 1 and 2 are to be related to group 3 rather than to group 4, an interpretation that may be reconciled with the cytological and genetic data if group 3 and *subobscura* are supposed to represent independent origins of a rod-shaped X. It may be noted that *subobscura* does not appear to be as similar to the members of group 3 (in characters other than chromosomes) as do the members of the *affinis* sub-group. The closest intergroup resemblances here are betweeen the members of the *affinis* and *melanogaster* sub-groups.

The diagram may be modified as shown below, in order to produce a compromise between the two sets of characters—chromosomal and non-cytological. This scheme is, naturally, to be taken only as a working model. The study of additional species, especially from the eastern Palaearctic region, is perhaps the greatest present desideratum for its improvement. (No member of group 4 has been seen from the area between Moscow and Alaska.)



If the diagram is accepted as giving an approximate picture of the phylogeny of the subgenus, the next question that arises is—what is the point of origin? This question may be approached in several ways. One method is through comparative cytology. Of the various chromosome configurations shown, the ones that are known outside of the subgenus are:

melanogaster type—in *Chymomyza amoena*, *Scaptomyza graminum*, and (with doubt as to which chromosome is the X) in *Mycodrosophila dimidiata*, *Chymomyza procnemis*, *Chaetopsis fulvifrons*, and the following species of Drosophila (all of uncertain subgeneric reference, but probably neither Sophophora nor subgenus Drosophila): *bromeliae*, *florae*, *coracina*. *Quinaria* and *robusta*, of the subgenus Drosophila, have been referred here, but *robusta* is certainly different, and the published figures for *quinaria* show no dot and do not indicate which is X.

subobscura type—in *ramsdeni*, *virilis*, *similis*, *cardini*, *mulleri*, *phalerata*, *transversa*, all belonging to the subgenus Drosophila. Essentially the same arrangement, with differences only in amount and distribution of heterochromatin, occurs also in the following members of the subgenus Drosophila: *funebris*, *histrio*, *hydei*, *repleta*.

pseudoobscura type—in *virilis americana* (HUGHES 1939), where it is evidently a secondary derivative of the *subobscura* type.

affinis type—in *duncani* (DOBZHANSKY, unpublished), which is the type of an unnamed subgenus (referred by STURTEVANT (1939) to Dasydrosophila Duda, but not in reasonable agreement with the type of that subgenus).

This comparison thus suggests that species-group 3 is related to Chymomyza and Scaptomyza and less certainly to Chaetopsis (of the very remote family Ulidiidae), Mycodrosophila, and two little-understood subgenera of Drosophila; and that species-group 4 is related to the subgenus Drosophila and to *duncani*. There is no indication that groups 1 and 2 are connected directly with any other form that has been studied.

The method of comparing resemblances in many different characters (STURTEVANT 1939) is in general agreement with this. The closest resemblance of any species of Sophophora with any of the other species examined is that between the *affinis* forms and an undescribed member of the subgenus Drosophila from the mountains of California. It should be pointed out that none of the forms listed above as having the *melanogaster* group is included in these latter comparisons. An undescribed American species that is close to *coracina* is included; it does not have the *melanogaster* chromosome group, and does not appear to be closely related to *duncani* or to any of the species of Sophophora or Drosophila that have been examined.

Another method of approach is through examination of the characteristics of each species group, to determine which seem most likely to be special and recently arisen peculiarities. In this category of characters are to be placed the opaque areas on the tergites (group 1), and sex-combs

(groups 3 and 4). It is also rather probable that relatively short ventral receptacles and testes (group 4, and group 3 to a lesser degree) are primitive. This method may be taken as agreeing with the others in indicating that group 1 is not the point of origin for Sophophora, and in suggesting group 4 as the more primitive—though group 2 is also to be considered as a possibility.

The most obvious inter-subgeneric relationship is that between group 4 and the subgenus Drosophila. It should be pointed out that Drosophila is a more complex subgenus than Sophophora, including more species groups, whose interrelationships and geographical distribution are more difficult to describe. It seems likely, therefore, that Drosophila is at least as old as Sophophora—again suggesting group 4 as the most ancient one in Sophophora.

SUMMARY

1. Forty-seven mutant types of *Drosophila affinis* are described, dependent on mutant genes in 40 different loci. Of these, 13 are in the X chromosome, 15 in the second, 5 in the third, 4 in the fourth, and 3 in the fifth.

2. Twenty-eight of these types resemble ones previously known in *D. melanogaster* or *D. pseudoobscura*. Their distribution among the chromosomes indicates the following homologies:

affinis	XL	XR	2	3	4	5
pseudoobscura	XL	XR	2	3	4	5
melanogaster	X	3L	3R	2R	2L	4

3. There are no probable parallels in disagreement with these homologies.

4. The subgenus Sophophora falls into four species-groups. A discussion of the morphology and cytology of the available representatives of each of these groups leads to a tentative phylogeny. The species-group that includes *pseudoobscura* and *affinis* is perhaps nearer the ancestral type than are the other three. This leads to the supposition that the *melanogaster* chromosome group was derived from the *pseudoobscura-affinis* arrangement.

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AN ANALYSIS OF THE BRISTLE FORMING REACTIONS IN DROSOPHILA MELANOGASTER SCUTE-1

GEORGE CHILD
Amherst College, Amherst, Massachusetts

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INTRODUCTION

THAT portion of development during which a change in temperature produces a differential effect on the expression of some character is the temperature-effective period (TEP) for that character. Such periods have been determined for several characters of *D. melanogaster*, HOGE (1915), KRAFKA (1924), DRIVER (1931), E. DRIVER (1925, 1931), LUCE (1931), PLUNKETT (1926), STANLEY (1932), and HARNLY (1932, 1936). Some of these workers have attempted to explain the mechanism of the temperature effect by the form of the curves obtained when the values of the measured character are plotted against the time of transfer. It is significant that the form of these curves, in all cases, appears to be sigmoid, resembling to some extent the probability integral curve. CHILD (1935b) and MARGOLIS (1935) have suggested that the form of these curves for the bristles of scute-1 and the facets of Bar eye, respectively, may be determined chiefly by the distribution in time of the TEP in the individual flies. In other words larvae hatching from eggs laid at the same time do not all develop at the same rate and hence enter their individual TEP's at different times. The TEP of the population (P) is the summation of two factors 1) the variation (V) among the larvae in time of entering the TEP and 2) the duration (D) of the TEP in the individual flies, $P = V + D$. It was shown that V could be represented by a normal frequency distribution.

In this paper the data are analyzed further to obtain the curves and equations of the developmental process leading to bristle number determination in scute-1. Using these equations and the curve of the probability integral it is further possible to calculate the P curve.

EXPERIMENTAL

The culture methods and the tests for homogeneity of the stock were reported previously (CHILD 1935a). One and two hour egg-laying periods were used. Groups of developing flies were transferred from 28° to 20° during P and larvae were removed to agar slants at two-hour intervals as they pupated. The mean bristle numbers of these pupating groups were recorded separately at eclosion. A number of such experiments were conducted, the transfers being made at various times during P. Table 1 shows the results on the ocellar (oc) and anterior notopleural (anp) bristles for the females. The bristle numbers are expressed as mean per half fly, for

TABLE I

♀♀

Relation between bristle number and time at which flies pupate after being transferred from 28° to 20° during P at time indicated in column heading.

82 HOURS				86 HOURS				87.5 HOURS			
t.	NO.	MOC	MANP	t.	NO.	MOC	MANP	t.	NO.	MOC	MANP
96	1	1.0	0.5	88	1	1.0	0	85.5	1	1.0	0.5
98	1	0.5	0.5	90	0			87.5	2	0.75	0.75
100	*2	0	1.0	92	3	1.0	0.83	89.5	4	0.62	0.62
102	1	0	0	94	3	0.66	1.0	91.5	0		
104	13	0	0.04	96	2	0.75	1.0	93.5	8	0.75	0.44
106	17	0.06	0.06	98	*8	0.19	0.75	95.5	24	0.62	0.60
108	24	0.02	0	100	32	0.14	0.42	97.5	*54	0.36	0.55
110	37	0	0.02	102	32	0.03	0.43	99.5	80	0.32	0.46
112	30	0	0.02	104	45	0.11	0.21	101.5	73	0.16	0.33
114	44	0	0	106	7	0	0.14	103.5	74	0.07	0.26
116	30	0	0	108	9	0	0	105.5	79	0.05	0.22
118	25	0	0	110	7	0	0	107.5	60	0	0.07
120	13	0	0	112	5	0	0	109.5	53	0	0.04
122	9	0	0	114	7	0	0	111.5	26	0	0
124	9	0	0	116	4	0	0	113.5	16	0	0
126	6	0	0	118	3	0	0	115.5	14	0	0
128	9	0	0								
130	6	0	0								
96 HOURS				93 HOURS				88.5 HOURS			
86	3	0.66	0.83	85	1	1.0	1.0	86.5			
88	5	0.7	0.5	87	3	0.83	0.5	88.5	5	1.0	0.6
90	8	0.5	0.44	89	4	0.63	0.63	90.5	2	0.75	1.0
92	16	0.63	0.61	91	8	0.56	0.56	92.5	7	0.57	0.5
94	32	0.61	0.71	93	12	0.83	0.46	94.5	17	0.56	0.91
96	19	0.58	0.65	95	18	0.67	0.69	96.5	*23	0.46	0.61
98	10	0.9	0.65	97	35	0.59	0.56	98.5	22	0.16	0.63
100	17	0.50	0.61	99	20	0.59	0.63	100.5	17	0.29	0.41
102	16	0.66	0.59	101	*39	0.36	0.49	102.5	9	0.22	0.55
104	*14	0.25	0.65	103	33	0.29	0.5	104.5	5	0.10	0.4
106	8	0.37	0.69	105	33	0.17	0.3	106.5	13	0.00	0.38
108	13	0.15	0.65	107	23	0.17	0.28	108.5	5	0.0	0.10
110	8	0.19	0.31	109	23	0.09	0.11	110.5	2	0	0
112	1		0.5	111	9	0.11	0.16	112.5	2	0	0
114	2		0.5	113	14	0	0.04	114.5	2	0	0
116	1		0	115	0						
				117	0						
				119	22	0	0				

example, if among 100 flies 30 ocellar bristles were found the Moc=0.15. Similar results were obtained for males.

The flies pupating in the successive two-hour intervals from each transfer period may be classified into three groups on the basis of mean bristle

number: 1) those having passed through D at the time of transfer; 2 (those still in D at the time of transfer; 3) those having not as yet entered D at the time of transfer. Groups 1 and 3 measure the end and the beginning of D, respectively. The mean bristle numbers of group 2 when plotted against time of pupation measures the course of the reaction during D.

TABLE 2

Larvae transferred from 28° to 20°. Pupae removed at two hour intervals and mean bristle number per half fly determined for each group at eclosion.

	NO. ♀♀ FLIES PUPATED			NO. ♂♂ FLIES PUPATED		
		MOC.	MANP.		MOC.	MANP.
28° control	760	0.60	0.46	685	0.41	0.037
	3	0.66	0.83			
	6	0.75	0.58			
	11	0.64	0.50	81	0.44	0.037
	21	0.66	0.48	184	0.47	0.038
	43	0.63	0.63	143	0.41	0.051
	40	0.71	0.51	86	0.55	0.041
	33	0.75	0.47	158	0.54	0.050
	71	0.59	0.53	186	0.44	0.038
	80	0.62	0.56	168	0.23	0.044
	140	0.35	0.55	135	0.13	0.037
	176	0.26	0.49	139	0.050	0.043
	181	0.14	0.36	88	0.011	0.023
	176	0.090	0.30	51	0	0.010
	139	0.051	0.18	34	0	0.014
	130	0.008	0.092	23	0	0
	110	0	0.032	17	0	0
	77	0	0	21	0	0
	55	0	0	14	0	0
	67	0	0			
	16	0	0			
	9	0	0			
	9	0	0			
	6	0	0			
	9	0	0			
	6	0	0			
20° Control	605	0	0	619	0	0

Unfortunately there are not enough flies in group 2 from each separate transfer period and it is therefore necessary to summate all the data. The data could be added together by making the time of transfer the starting time for each transfer period. This was not done since the results show that the TEP's in the individual flies do not begin at the same time in relation to the time of pupation in the various transfer groups. It is not possible, therefore, to use time of transfer as the zero time point in summating the data. These data were added together after aligning the pupat-

ing periods in which the change in temperature produced a noticeable effect on the bristle number. These periods are indicated by an asterisk. In other words, the beginning of D was determined for each group separately and then all the data were added together on this basis. The results are given in table 2. The control values for flies raised at 20° and 28° for their whole developmental periods are also given. The two hour periods between each group are at 20°. To convert this to time at 28° it is necessary to use the factor 94.1/185.4 and 89.6/178.6 for the females and males respectively. These figures are the durations in hours of the egg-larval periods at 28° and 20°.

ANALYSIS OF THE DATA

Before attempting an analysis of the data it is necessary to consider a fundamental assumption which must be made concerning the "all or none" behavior of a particular bristle in one fly and its relation to the mean bristle number of that bristle in the whole population. It is to be assumed that the mean bristle number of a particular bristle in a population of flies, uniform as to genetic and environmental factors, is proportional to the amount of bristle substance or precursor (for that bristle) present in that population at the time of bristle determination. This assumption implies that 1) there is a distribution in the amount of bristle substance among the flies, 2) a bristle will be present if the concentration of bristle substance exceeds a 'threshold' concentration and absent if below this threshold, 3) the value of this threshold and the nature of the distribution are such as to allow the mean of the distribution to be proportional to the mean bristle number. At the present time I can see no clear mathematical proof for this fundamental assumption. The fact that the data treated in this manner yield conclusions from which other results may be predicted makes the assumption necessary. PLUNKETT (1926) has also utilized this assumption in his analysis of the Dichaete gene and has considered its implications in greater detail than has been done here.

It has been shown (CHILD 1935a) that this scute-1 stock does not develop any ocellar or anterior notopleural bristles below 22°. It may be assumed therefore that below this temperature the reaction producing "bristle substance" goes on at a very slow rate and may be neglected. The data, on the basis of these assumptions describe the formation of "bristle substance" or precursor at 28°.

The beginning and the end of the reactions are taken as the periods in which the pupating groups give bristle numbers equal to the controls at 20° and 28° respectively. Only the oc and anp bristles are under consideration since there are few data on the other bristles.

Unsuccessful attempts were made to fit these data into a uni- or bimolecular reaction system. The mean bristle numbers rise rapidly indicat-

ing a logarithmic or exponential system. Plotting log time versus log bristle number (figure 1) gives reasonably good results. The straight lines were determined by the method of least squares. The anp male line is not satisfactory since the first point is based on only 34 flies and is not very significant. The other three points describe a line which would be more in harmony with the other bristle data. The equation for these log-log plots, log bristle number = k log time + constant, is essentially the type of equa-

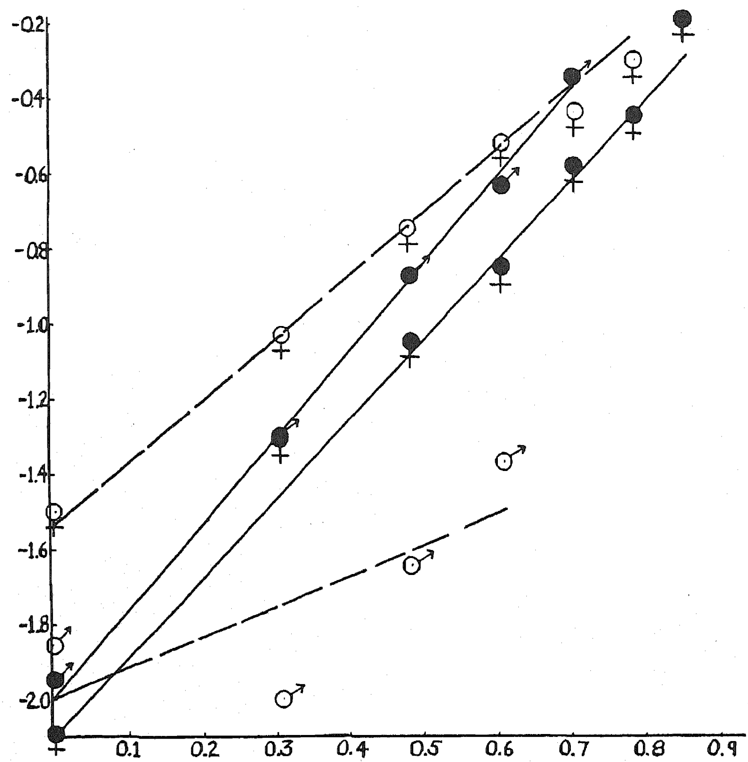

FIGURE 1.—The bristle reaction, log mean bristle number = k log time + constant. Ordinates, log mean bristle number; abscissae, log time o = anp, ● = oc.

tion from which HUXLEY (1932) derives his law of heterogonic growth. The differential form of this equation db/dt = k(b/t) implies that the rate of formation of bristle substance at time t is proportional to the average rate up to that time. This equation has been used by a number of investigators to describe organic growth (see NEEDHAM 1931). GLASER (1938) using another form of the equation dw/dt = k(dt/2t+1) applied it with considerable success to many categories of growth data (w = weight of the organism or some part of it). It may be mentioned here that since the log oc and log anp are both related to log t, plotting log oc versus log anp

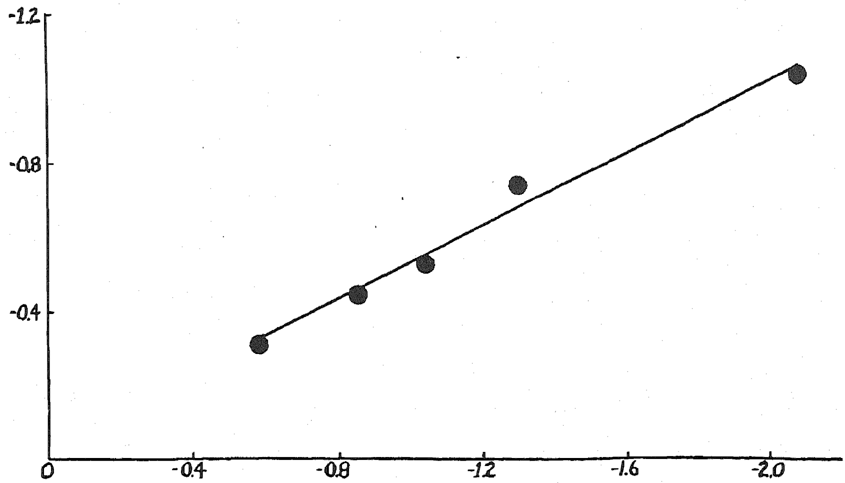

FIGURE 2.—The heterogonic relation between the bristles. Ordinates, log oc, abscissae, log anp.

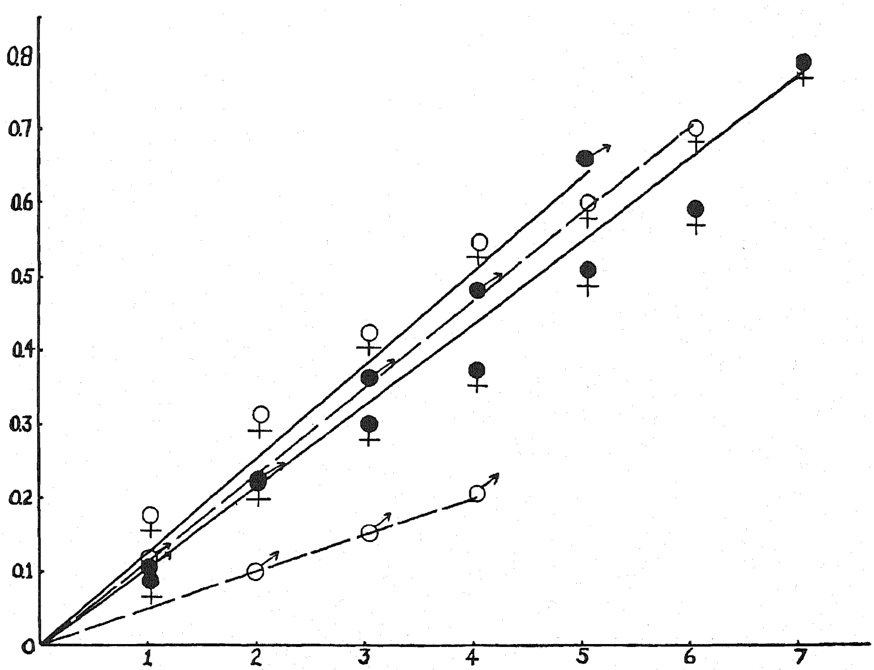

FIGURE 3.—The bristle reaction, mean bristle number$^{1/2}$ = constant × time. Ordinates, bristle number$^{1/2}$; abscissae, time. o = anp, ● = oc.

should give a straight line. Figure 2 shows that this is the case. This establishes a heterogonic relationship between the oc and anp bristles. NEEL

(1940) found that bristle number and body weight in his experiments are also related in this manner.

Too much significance, however, should not be attached to these equations since they do not adequately describe the kinetics of the bristle reaction and other empirical equations would do as well. The expression mean bristle number$^{1/2}$ = constant × time which is simpler than the log-log equation also appears to fit the data (figure 3). The slopes of these lines were calculated by dividing the durations of the reactions by the square root of the mean bristle numbers of the controls at 28°.

ANALYSIS OF BRISTLE REACTIONS

The lines show that the "rates of bristle formation" are not the same. The duration of the oc reaction is about one hour longer than that of the anp in both sexes. The reactions are about two hours shorter in the males than in the females. The oc reaction also starts earlier than the anp as can be seen from the table. Thus the anp male reaction lasts about four hours, the oc male five hours, anp female six hours and the oc female seven hours. The characters, mean bristle numbers are, on this basis, the results of differences in rates and durations of bristle reactions for the oc and anp bristles and in the two sexes. This type of generalization regarding characters has been made by many investigators with few definite measurements on the rates and durations of the developmental reactions (see Goldschmidt 1938). The analysis of the bristle numbers gives definite values to these parameters.

The analysis does not show, however, what the nature of the bristle forming reaction may be. Since at this larval period there are still no visible signs of bristles (Robertson 1936), the reactions at this period are probably not on the morphological level but must be on a chemo-embryological level. That is, actual bristle formation is not involved. The change in the amount of bristle precursor is being affected during this period of development.

The equations may give a clue to the kinetics of the bristle precursor reactions. The differential form of $b^{1/2} = ct$ is $db/dt = 2cb^{1/2}$. The other equation $db/dt = k(b/t)$ is identical with $db/dt = ak(b)^{1-1/k}$ in which a is a constant obtained by integration. These equations are similar in form to the types of equations found in the study of heterogeneous chemical reactions involving adsorption that is, $dp/dt = kp^n$ in which p is the pressure of a gas being adsorbed before it can react. The constant n is usually between 0 (zero order) and one (unimolecular) and in many instances is equal to $\frac{1}{2}$. Some of the reactions in which $n = \frac{1}{2}$ have been interpreted as diffusions.

On the basis of this analogy a number of mechanisms may be suggested

for the bristle substance reaction. The following analysis indicates one possible type of mechanism. In the early development of the larva, bristle substances are being synthesized in reactions which are not differentially affected by the presence of the scute-1 gene or its byproducts, that is, these reactions are the same in all respects as the reactions in the wild-type fly. As development proceeds to a certain time and stage, the processes which are affected by the presence of scute-1 (or its wild-type allele) begin, that is, these reactions are not the same in all respects as their homologous reactions in the wild-type fly. The time when the reactions begin, is the beginning of D. These "scute-1 controlled" processes may be an adsorption or diffusion of substances from a site of synthesis to a place at which future essential bristle forming reactions will occur. These diffusion or adsorption reactions cease at a time corresponding to the end of D. For the remainder of development or at least up to the time at which the presence or absence of a bristle is finally determined, the bristle reactions are the same in both wild-type and scute fly. These reactions, however, are affected by the amount of substrate received during the scute-1 reaction so that the bristle number of the adult scute-1 fly has been determined essentially by the scute controlled reaction.

It is common knowledge that the bristle numbers of the wild type are not affected by temperature changes (within this normal temperature range) during development. The results of IVES (1939) and of CHILD, BLANC and PLOUGH (1940) show, however, that bristle reactions take place in the wild-type fly during the period comparable to D in scute-1. If these diffusion or adsorption reactions do occur in the wild-type fly they must have higher velocity constants or longer durations to bring about a full bristle number in all flies. The temperature coefficients of these wild-type bristle reactions during D must also be in harmony with that of other developmental reactions so that the rate or durations of the bristle reactions may result in full bristle numbers at all temperatures. In temperature coefficient these reactions in the scute-1 fly, however, are not in step with the rest of the developmental processes so that scute-1 flies raised at different temperatures have rates of D which do not bear the same relations to the rates of other developmental processes at all temperatures. This differential effect of temperature in the scute-1 fly results in a change in bristle number at different temperatures. It further permits of the use of temperature to measure the rate, duration and temperature coefficient of the bristle reaction which differentiates between the presence of scute-1 or its wild-type allele in a developing fly. It is evident that the developmental processes leading to bristle formation are made less efficient by the substitution of scute-1 for the wild-type allele.

CALCULATION OF THE P CURVE FROM V AND D

As mentioned previously, the variation in time at which the larvae enter their temperature effective periods follows the probability integral curve. At 28° the practical limits of this curve ($V \pm 3\sigma$) are approximately 18 hours. Taking the oc bristles in the female as a type case, the P curve may be calculated from V and curve of D. From a probability table or curve it is seen that in a population transferred 1σ or three hours after the beginning of P 97.7 percent of the larvae have not entered their TEP, 1.9 percent have been in it for 0.5 hours and .4 percent for 2 hours. From the equations of D the bristle numbers of these groups may be determined. The average of this population is 0.0003. In a population transferred at

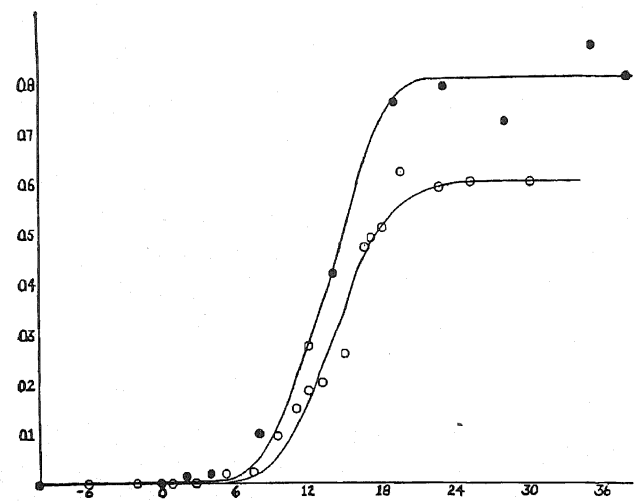

FIGURE 4.—Calculated P curves. Experimental points obtained by measuring the bristle numbers of populations of flies transferred from T_1 to T_2 during development. Ordinates, mean oc ♀♀ bristle numbers: abscissae, time in hours from the beginning of P. o $= T_1 28°$, $T_2 28°$; ● $= T_1 29.7°$, $T_2 22.0°$.

2σ or six hours 84 percent have not entered, the periods for the remaining 16 percent obtained from V and their bristles numbers from D. The average is taken and this gives the six hour point on the P curve. In a similar manner points are obtained for 9, 12, 15 and 18 hours. Figure 4 shows the smooth curve obtained from this calculation. When the experimental values obtained from many earlier experiments (CHILD 1935b) are superimposed on this line as in figure 4 it can be seen that there is reasonable agreement. In a similar manner the P curves for the other bristles have been calculated. It follows from these calculations that P can be obtained from V+D and that interpretations of the nature of the developmental processes leading to character formation cannot be made from curves of P. If the TEP is to be studied the D curve must be determined.

CALCULATION OF P AND D AT OTHER TEMPERATURES

To calculate the curves of D and P at other temperatures it is necessary to assume that the durations of D and V are proportional to the time of development at these temperatures. This is not unreasonable since it was shown (CHILD 1935b) that the duration of P is approximately proportional to the time of development. The mean bristle number and the time of development have been previously reported (CHILD 1935a, b). Substituting these values in the equations $\log b = k \log t + \log a$ (assume constant a) and $b^{1/2} = c^t$, the equations for the curves of D at these temperatures may

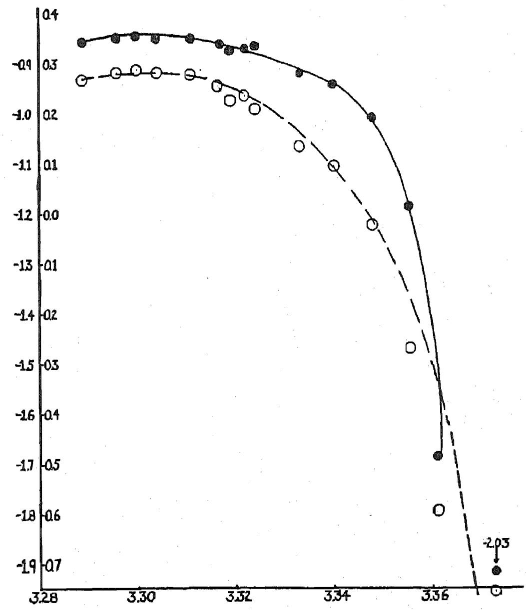

FIGURE 5.—Relation between temperature and velocity constants of the bristle reaction. Ordinates, inner scale (and solid line) log k from log-log equation, outer scale (and broken line) log c from half-power equation; abscissae, reciprocal absolute temperature $\times 10^{-3}$.

be obtained. Since k and c represent "velocity constants," it was of interest to determine whether they were related to temperature as in the Arrhenius equation $d \log k/dT = u/RT^2$. In plotting log c and log k against $1/T$ (figure 5) straight lines are not obtained, that is, u is not constant. This is not surprising, however, since very few biological reactions have constant temperature coefficients. These curves, however, give the values of k and c at all temperatures. By substituting these values into the D equations the course of a bristle reaction may be described at all temperatures. Using the curves of V and these curves D as in previous calculation for P at 28°, the curves of P at all temperatures may be calculated. Figure

4 shows the curve of P for transfers between 29.7° and 22°. The experimental points are too few to make a reasonable comparison with the calculated curve.

SUMMARY

The sigmoid curves (P) which are usually obtained in the determination of temperature-effective periods are the sum of at least two curves resulting from, 1) the variation (V) from fly to fly in the time at which their temperature-effective periods begin and, 2) the rate and duration (D) of the affected reaction in the individual flies. The separate curves of V and D have been obtained for scute-1 by an elaboration of the usual methods for determining temperature-effective periods The results indicate that in a population of developing flies obtained from an egg-laying period of 1–2 hours the variations in the time at which the larvae enter their sensitive periods follow a normal distribution curve.

The curves showing the rates and durations of the various bristle forming reactions are also obtained from these data. An analysis of the D curves indicates that they fit the equation $\log b = k \log t + \text{constant}$ where b = mean bristle number per half fly, t = time, and k is a constant. The constants and the durations of the reactions are not the same for the various bristles. The data also fit other similar equations including a simple form $b^{1/2} = ct$. These equations are similar to the equations found in heterogeneous chemical reactions. The kinetics of the scute-1 reaction are discussed from this point of view. Using the curves of D and V it is possible to calculate the P curves. Interpretations of the mechanism of developmental processes therefore cannot be made from curves measuring the temperature effective period of the population but require a study of the reactions in the individual fly.

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GAMETIC LETHALS ON THE FOURTH CHROMOSOME OF MAIZE*

W. RALPH SINGLETON AND P. C. MANGELSDORF

Connecticut Agricultural Experiment Station, New Haven, Connecticut, and Texas Agricultural Experiment Station, College Station, Texas

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PLANTS of maize, heterozygous for *su*, have been observed that produced no *su* seeds when selfed or pollinated by *su* pollen, but whose pollen when applied to *su* ears produced in excess of 96 percent *su* seeds (figure 2, H, I). This anomalous condition is caused by two gametic lethals, small pollen-1, (*sp*), and lethal ovule-1 (*lo*).[1] Preliminary reports on the inheritance of these two factors have been published, (MANGELSDORF 1931, 1932, SINGLETON 1932). Both *sp* and *lo* are closely linked with *su*(*sp su* = 5 percent recombinations; *su lo* = 2.2 percent) and give distorted ratios of *Su*:*su* because of this close linkage and the fact that one gene is lethal to the male, the other to the female gametes. Pollen grains of the composition *sp* are smaller than normal (figure 5, C, D, and E) and in most cases do not function in competition with normal grains. Ovules carrying *lo* usually abort before fertilization and are rarely capable of being fertilized. The ears referred to above were of the composition *sp*++/+*su lo*. The only functional ovules were of the composition *Lo* which were mostly *Su*, in a few cases all *Su*, when the crossing over may have been reduced or due to random sampling an occasional ear was all *Su*. The functional pollen was *Sp* in constitution and was *su* with the exception of recombinations.

ORIGIN OF *sp* AND *lo*

The gametic lethal *sp* was discovered by the junior author while on the staff of the Connecticut Experiment Station in 1924. This factor must have arisen as a mutation in a Leaming strain, Connecticut 112–8, that had been inbred four generations. It is not possible to say in what generation the *sp* factor arose although it is practically certain to have occurred after the first generation, since the original ear selfed once showed no missing kernels, a characteristic of *sp*/+ ears. This condition, when it first appeared, was in the repulsion phase, *sp* linked with *Su*, which accounts for the high percentage of surgary seeds when selfed or backcrossed either way.

The *lo* factor arose in a stock segregating for *sp* and *su*. It must have arisen as a mutation on the homologue of the chromosome carrying *sp*,

* The cost of the accompanying half-tone illustrations has been borne by the Galton and Mendel Memorial Fund.

[1] Factor symbols not accompanied by numerals represent either the first or the only gene with that literal symbol; that is, $sp = sp_1$, etc.

since *lo* was linked with *su* when first observed while *sp* was linked with *Su*. The most probable explanation of the origin of *lo* is that its occurrence in a stock segregating for *sp* was purely a coincidence although there is a possibility of a causal relationship. The first *lo*/+ ear had 11.1 percent of *su* seeds. The two preceding generations had 53 and 64 percents respectively, showing presence of the *sp* factor but absence of the *lo* factor. All studies of the *lo* condition were made by the senior author in Connecticut. The inheritance of this factor was complicated at first because the stock was also segregating *sp* and gave the unusual results described above. However the two factors have been separated and each studied independently and in combination.

INHERITANCE OF *sp* AND *lo*

Disturbance of su ratios

Both the factors *sp* and *lo* were discovered because of their close linkage to *su* and the inheritance of each has been studied largely through its disturbance of the *su* ratio in selfed and backcrossed ears. The *sp* condition was originally termed "high sugary" since it was in the repulsion phase, *sp*+/+*su*, and gave a high percentage of *su* kernels (figure 1).

The other gametic lethal, *lo*, was originally called "low sugary" because the coupling phase, *lo su*/++, the one first observed, gave a very low percentage of *su* kernels. The factor *sp*, produces extremely low sugary ratios when in the coupling phase *sp su*/++, while the original "low sugary," *lo*, produces very high sugary ratios in the repulsion phase *lo*+/+*su* (figure 2).

The disturbance of the *su* ratio in stocks heterozygous for *lo* or *sp* can perhaps best be understood by presenting in tabular form the functional male and female gametes. This is presented in table 1.

TABLE 1

Functional gametes in plants segregating for sp, lo, or sp and lo not counting double-crossovers or survival of sp ♂ gametes or lo ♀ gametes.

GENOTYPE	FUNCTIONAL GAMETES ♀	FUNCTIONAL GAMETES ♂
sp +/+ *su*	*sp* +, + *su* *sp su*, ++	+ *su*, ++
lo su/++	++ + *su*	*lo su* + + *lo* +, + *su*
sp su +/++ *lo*	*sp su* + + *su* + + + +	+ + *lo* + *su* + + + +

Linkage relations of sp with su

Since in preliminary trials it was found that *sp* functions quite rarely (about one percent of the progeny plants were *Sp sp;* MANGELSDORF 1932) in competition with normal pollen, the backcross of *su su* ears by pollen from *sp*/+ plants has been used to give the crossover percent directly. Table 2 gives the total counts of all self pollinations or back-crosses in Connecticut and Texas.

TABLE 2

Percentages of su seeds obtained for selfed ears, and backcrosses to su for stocks segregating for sp, lo, or sp and lo.

GENOTYPE	SELF TOTAL	SELF % *su*	TIMES *su* TOTAL	TIMES *su* % *su*	POLLEN ON *su* TOTAL	POLLEN ON *su* % *su*	EXPECTED SELF RATIO (5×7)	DIFFERENCE (3–8)
1	2	3	4	5	6	7	8	9
sp +/+ *su*	143M	60.8	36M	61.9	158M	93.9	58.1	+2.7
sp su/++	7M	3.2	6M	37.6	41M	9.7	3.6	− .4
lo su/++	31M	1.8	54M	2.4	18M	51.3	1.2	+ .6
lo +/+ *su*	8M	47.1	27M	95.2	34M	48.5	46.2	+ .9
sp ++/+ *su lo*	7M	5.2	1M	8.5	14M	91.6	7.8	−2.6
++ *lo*/*sp su* +	2M	4.8			15M	26.5*		

* Only 28.6 percent of these are recombinations (true C.O. value = 7.6) remainder represent functional *sp* male gametes.

The percent of *Su* seeds, 6.1, in the backcross *su*×*sp*+/+*su* is the maximum average percentage of recombination between *sp* and *Su* in the repulsion phase, and represents the actual crossover value if the amount of functioning of *sp* pollen is negligible.

Functional *sp* pollen

Several cases of supposedly high crossover ratios have been found during the course of this investigation. In 1926 and 1927 the percents were 10.1 and 19.3 based on totals of five and one thousand seeds respectively. Also in 1932 several progenies were found that gave rather high recombination values. These could be explained either by an actual increase in the crossing over or by functioning of the linkage class of *sp Su* pollen grains. To test these two alternatives, some of the *Su* crossover seeds produced by the pollination *su*×*sp*+/+*su* were grown. Pollen was examined to determine whether normal plants or plants segregating *sp* had been obtained. The results of all such examinations are given in table 3. Certain facts are obvious in this table. Of a total of 2294 plants examined, 15.3 percent resulted from functioning *sp* pollen grains. This is considerably greater than the one percent previously reported. It may be argued that the value of 15.3 percent is too high since we were testing, in some cases, progenies

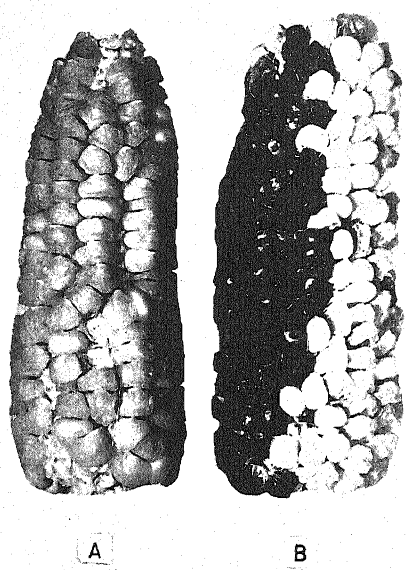

FIGURE 1.—Self pollination and backcross to *su* of *sp+/+su* plant. A -*su* times pollen from heterozygous plant; very small percent of *Su* seeds, the crossover class (11.6 percent). B shows a dual pollination, colorless seeds representing self pollinations, the black seeds being pollinated by *ACRPr su*. In making this pollination silks are divided approximately equally and different pollen applied to each half of the silks. Selfed seeds=58.0 percent *su*, ×*su*=66.1 percent *su*.

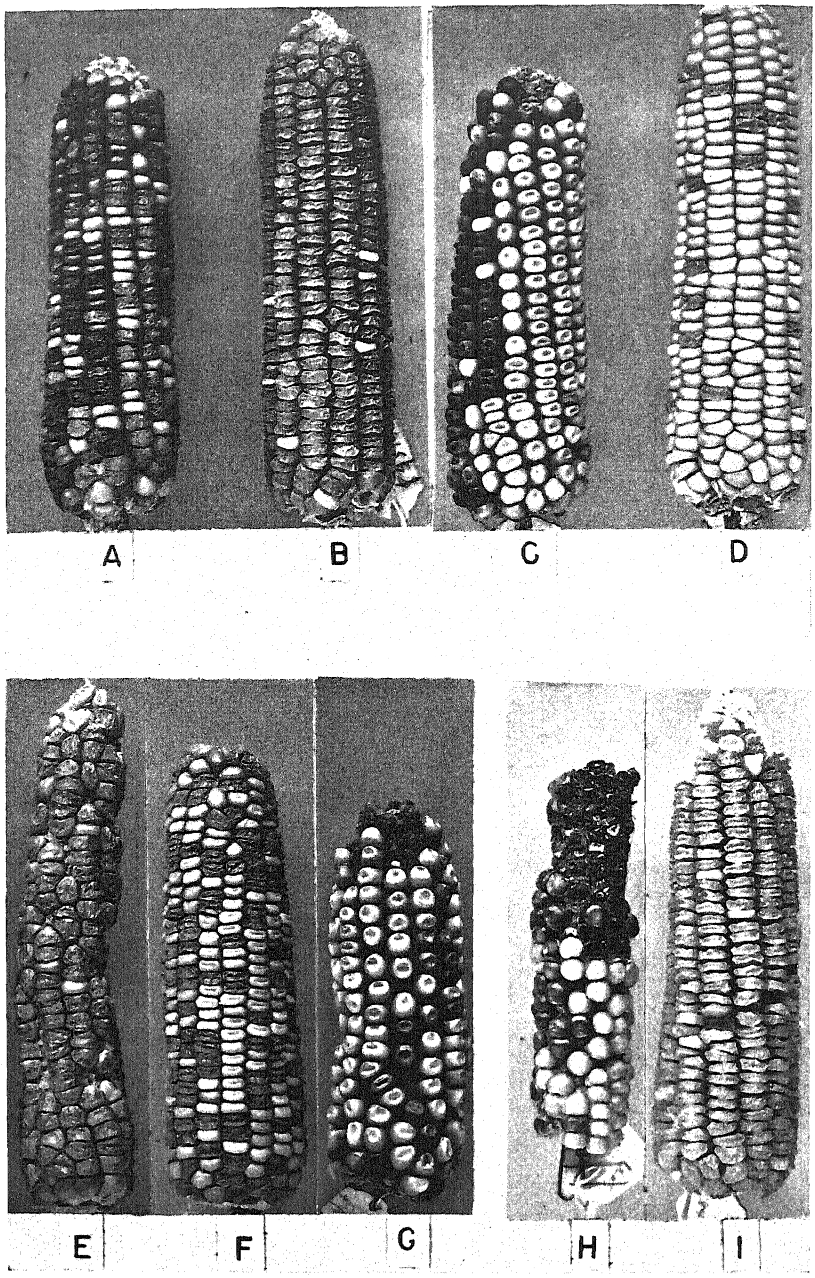

FIGURE 2.—A=ear from *sp+/+su* plant selfed (58.4 percent *su*), and crossed by *ACRPr su* (56.5 percent *su*); B=pollen from A put on *su* ear (97.1 percent *su*); C=*sp su/++* ear selfed (2.2 percent *su*) and crossed by *ACRPr su* (1.6 percent *su*); D=pollen of C on *su* (6.1 percent *su*); E=*lo+/+su* ear crossed by *su* (95.2 percent *su*); F=pollen of E on *su* (49.9 percent *su*); G=*lo su/++* ear selfed (0 *su*, 180 seeds) and crossed by *su* (0 *su*, 20 seeds); pollen when applied to *su* produced 49.8 percent *su*, ear similar to F. H represents *sp++/+su lo* ear selfed (0 *su*, 85 seeds), and crossed by *su* (0 *su*, 64 seeds); I=pollen of H on *su* (96.7 percent *su*).

TABLE 3

Percent of sp functioning pollen grains from the cross su×sp +/+ su.

	YEAR SEED COUNTS MADE	% IN C. O. CLASS	YEAR AND PLACE GROWN	TOTAL PLANTS EXAMINED	sp/+ NO.	sp/+ %	+/+ %	CORRECTED C. O. %
1	1927	9.8	1928 Texas	111	2	1.8	98.2	9.6
2	1926	13.3	1927 Conn.	227	2	6.4	93.6	12.4
3	1929	12.4	1930 Texas	89	0	0	100	12.4
4	1932	10.5	1934 Conn.	116	34	29	71	7.5
5	1932	4.8	1934 Conn.	526	111	21	79	3.8
6	1933	17.5	1935 Conn.	122	67	55	45	7.9
7	1933	8.6	1935 Conn.	1103	135	12	88	7.6
	Total and Average			2294	351	15.3	84.7	
		6.1*	(value for progenies 5 and 7)				84.9	5.2

* Average of all ears counted in Connecticut and Texas.

whose crossover values were obviously high. Two of the populations examined for segregating pollen represented random samples (progenies 5 and 7 in table 3). These two produced a total of 1629 plants of which 246 or 15.1 percent represented functional *sp* pollen instead of recombinations. This left a value of 84.9 percent for recombinations. When this is multiplied by the original crossover value of 6.1, it gives a corrected crossover value of 5.2 percent. This probably represents more nearly the correct value for the recombinations of *su* and *sp* in the repulsion phase.

Our correction factor was based on the number of *sp* pollen grains that not only effected fertilization but *produced mature plants*. Since our experiments have shown a differential viability in favor of *Sp Sp* plants, it is obvious that the number of *sp*/+ plants that survive to the flowering stage is appreciably lower than the seeds of the constitution *sp*+/+*su*. The number of plants lost is not usually known. Any appreciable increase in the number of *sp* functional pollen would further decrease the recombination percentage. In the 1932 ears, the original recombination percent was 4.8. When the crossover seeds were planted in 1935 and the resultant plants were examined for segregating pollen, 21 percent were segregating leaving 79 percent as recombinations. This gives a corrected crossover value of 3.8. This represented several progenies and probably approaches the true crossover value for those progenies.

We are forced to conclude that the original figure of 6.1 percent is slightly too high. Whether there is considerable fluctuation in the recombination percent as well as in the amount of functional *sp* pollen is not possible to determine since we have no accurate way of measuring the *sp/Sp* seeds that fail to produce plants. In some cases, at least, we know the value to be as low as four percent and it could be even lower. Since six percent

is the maximum, an average value of five may be taken as the crossover percent. A difference of one percent in the crossing over of these two genes will have little affect on the location of *sp* on the chromosome.

In the coupling phase, the percent of recombinations (plus *sp* pollen survivals) is 9.7 in a total of forty-one thousand. The apparently higher recombination value than for the repulsion phase is probably accounted for largely by *sp* pollen survivals rather than an increased crossover value. This will be discussed more fully under effects upon the male gametophyte.

Linkage relations of lo with su

Like *sp*, the gene *lo* is closely linked with the *su* locus so that its inheritance is studied through its effect on the *Su*:*su* ratios. In table 2 are found the apparent crossover ratios for *lo* and *su* in both the coupling and repulsion phases. Since *lo* ovules are nearly all lethal, the crossover ratio between *lo* and *su* is obtained by backcrossing *lo su*/++ or *lo*+/+ *su* ears by *su* pollen and noting the percentage of "crossover" seeds. The *su* seeds from the cross *lo su*/++ will all be crossovers provided there is no functioning of the *lo su* ovules. This point can be tested in a way similar to that used in testing *sp* survival in the pollen. The "crossover" seeds were grown and the resultant plants examined to determine how many produced *lo*/+ ears and were therefore *lo* ovule survivals instead of recombinations. Table 4 summarizes all "crossover" plants examined.

TABLE 4

Percentage of lo/+ plants produced by "crossover" seeds in both coupling and repulsion phases of lo and su.

GENOTYPE	YEAR	TOTAL PLANTS	*lo*/+ EARS NO.	*lo*/+ EARS %	% ++ EARS	ORIGINAL C. O.	CORRECTED C. O.
lo su/++	1932	88	15	17	83	2.3	1.9
lo su/++	1935	37	22	60	40	1.7	0.7
Total and av.		125	37	30	70	2.3	1.6
lo +/+ *su*	1935	318	220	69	31	5.1	1.6
lo su/+ *su*×++	1933	465	9	1.9*			

* = percentage survival of *lo* ovules compared to *Lo* when each given equal opportunity—no selection of seed (all *su*×*Su*).

In both the coupling and repulsion phases some of the apparent crossover seeds were *lo* ovule survivals. The seeds planted represented random samples of the crossover class. The apparent crossover ratio in the repulsion phase was greater than for coupling of *lo* and *su*. However there were more *lo* ovule survivals in the crossover seeds from the repulsion phases. When both crossover ratios from the progenies whose crossover seeds were examined are corrected, they are exactly the same, 1.6 percent in each case. The "crossover value" obtained in the coupling phase for all years studied

was 2.4 as given in table 2. When this is corrected by multiplying by 70 percent (see table 4) it becomes 1.7 percent, the actual crossover ratio for *lo* and *su* for all years grown. Likewise the total figure for the repulsion phase *lo*+/+*su*, 4.8, when corrected becomes 1.5 percent, the actual recombination percentage for *lo* and *su*.

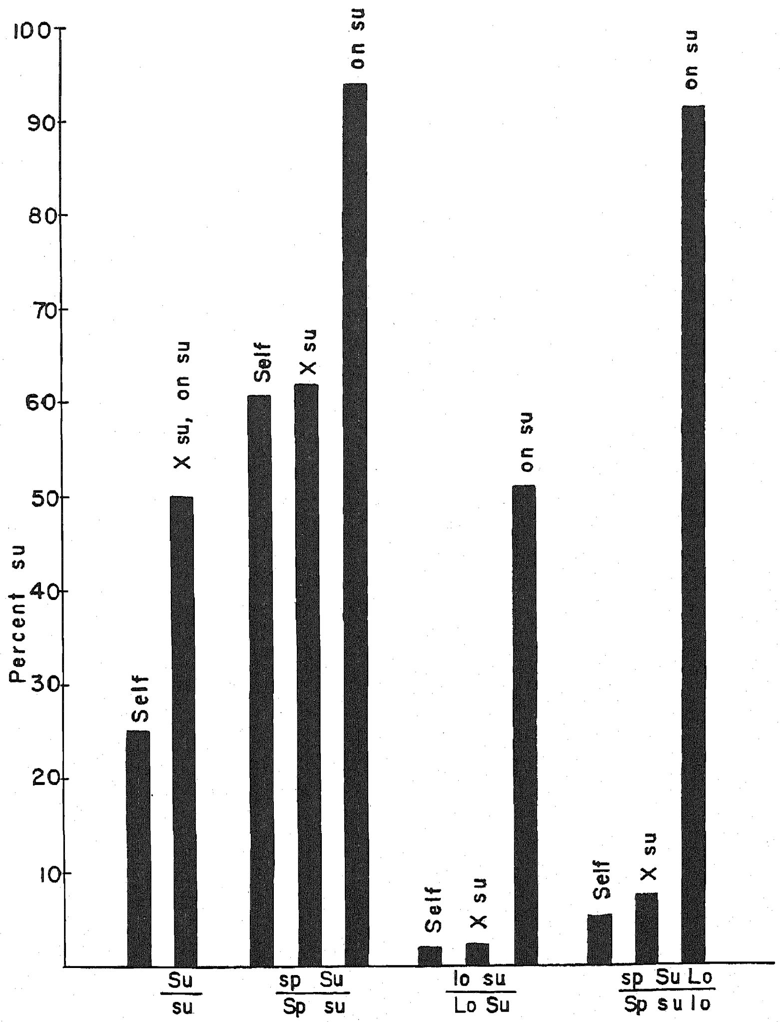

FIGURE 3.—Percent of *su* seeds obtained from self-pollinations and backcrosses to *su* for plants of the compositions *su*/+, *sp*+/+*su*, *lo su*/++ and *sp*++/+*su lo*.

Gametic and zygotic ratios of sp/+ and lo/+ plants

Table 2 gives the gametic ratios (backcrosses to *su*) and zygotic ratios (self-pollinations) produced by plants heterozygous for *sp*, *lo*, and combinations of *sp* and *lo*. This is also shown graphically in figure 3. From the

gametic ratios it is possible to calculate the expected zygotic ratio for comparison with the zygotic ratio found. The expected ratio is given in the eighth column of table 2. By comparing the actual and expected zygotic ratios it is possible to determine whether there is any appreciable differential zygotic mortality between heterozygotes and homozygotes. If there is a regular and appreciable survival of *sp* in the pollen or *lo* in the ovules, there should be some homozygotes produced. If these are eliminated the actual percentage of *su* seeds should be higher than the calculated percentage in the repulsion phase, and lower in the coupling phase. In all cases there is a fairly close agreement between the expected and observed ratios, the differences being noted in the last column of table 2. Some of the differences are negative and some positive. This would indicate no appreciable differential zygotic mortality. To test this point more thoroughly in the case of *sp+/+su*, plants from the same progenies were studied in Texas in 1930. Nine progenies were involved which included 255 ears with a total of 57,159 seeds. The total difference for the nine progenies between calculated and observed zygotic ratios was 2.3. In four cases the actual percentage was higher than the calculated and lower in five. All of these data show that differential zygotic mortality is not very influential in disturbing the ratios resulting from selfing heterozygous *sp* or *lo* plants. If there is any differential zygotic mortality it is overshadowed by errors in sampling, differences in crossing over at microsporogenesis and megasporogenesis, and differences in survival of *sp* male gametes.

EFFECTS OF *sp*

Effect on male gametophyte

The effect of *sp* on the male gametophyte was partially discussed in a previous section. The obvious effect is to cause the pollen grains to be reduced in size. In some samples it is possible to count the two classes of pollen grains and in such cases ratios of approximately 1:1 are found (626 normal:643 small in one sample counted). In others, however, there is an overlapping of the two classes. When the distribution of the pollen grains with respect to maximum diameter is plotted as a frequency polygon, the curve for pollen grains from *Sp Sp* plants has only one mode at 96 microns while the curve for *Sp sp* plants is distinctly bimodal with one mode at 84 microns, the other at 99 microns (figure 4).

Of some interest in this connection is the fact that the normal pollen grains from *Sp sp* plants are larger than the grains from normal plants. This is suggested by the frequency distribution in figure 4 and is verified by an actual comparison of all grains with a maximum diameter of 90 microns or more. In *Sp sp* plants these grains average 98.4 microns; in *Sp Sp* plants, 95.3 microns. The difference, 3.1 microns, is probably significant (P

<.03). Evidently the normal pollen grains in heterozygous plants are capable of taking advantage of the reduction in competition resulting from the fact that half of the pollen grains are reduced in size.

Microspores of plants segregating *sp* were examined to see how early the *sp* microspores could be detected. No difference in microspore size or appearance was found at the first microspore division. Just before the second division however, the *sp* grains are slightly smaller and the nucleolus of the tube nucleus is more vacuolated than that of *Sp* pollen grains (figure 5, C and D).

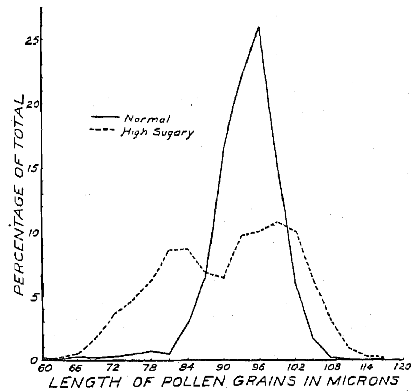

FIGURE 4.—Frequency distribution, with respect to length, of 1350 pollen grains from eleven $sp+/+su$ (high sugary) plants and 1100 grains from $su/+$ plants.

Although the *sp* grains are smaller than normal they are usually well filled with starch. Preliminary results showed that the *sp* grains rarely effect fertilization in competition with normal grains although they are capable of fertilization when the competition with normal grains is removed by screening (MANGELSDORF 1931, 1932). More recently it has been shown (SINGLETON 1940) that the pistillate parent to which the pollen is applied has considerable influence on the functioning of *sp* pollen. In 1938 the recombination percent of *su* and *sp* was high when pollen of $sp\ su/++$ was applied to *su*. Crosses on Purdue 39 gave 39.1 percent of *su* seeds, those on Connecticut 81 gave 16.4 percent. Both of these figures suggest that there was considerable functioning of *sp* pollen, more in the case of P39 than for C81. Examination of plants produced by these seeds was made in 1939. The results are given in table 5.

This table shows that the *su* seeds from the P39 cross produced 86.8 percent of $sp/+$ plants while the C81 crosses gave only 55 percent. The corrected crossover ratio for the P39 crosses was 5.2 whereas for the C81 crosses it was 7.4 percent. This difference cannot be a difference in crossing over since pollinations on the two inbreds were made on the same day

TABLE 5

The effect of the pistillate parent upon survival of sp in pollen. Crosses of P39 and C81 by sp su/+ + plants.

PARENTS		NO. SEEDS			PROGENY PLANTS		%	%	CORRECTED
♀	♂	TOTAL	*su*	%*su*	TOTAL	*Sp sp*	*sp*/+	++	C. O.
P39	1174-4	1612	607	37.7	123	111	90.2	9.8	3.7
C81	1174-4	1135	115	10.1	47	32	68.1	31.9	3.2
P39	1175-10	1382	728	52.7	183	165	90.2	9.8	5.2
C81	1175-10	2327	434	18.7	127	58	45.7	54.3	10.2
P39	1176-5	600	156	26.0	114	93	81.6	18.4	4.8
C81	1176-5	1997	350	17.5	91	62	68.1	31.9	5.6
P39	1176-8	864	253	29.3	95	78	82.1	17.9	5.2
C81	1176-8	1365	223	16.3	86	41	47.7	52.3	8.5
Total on P39		4458	1744	39.1	515	447	86.8	13.2	5.2
Total on C81		6824	1122	16.4	351	193	55.0	45.0	7.4

using pollen from the same plants. These results suggest that there might have been a more selective elimination of heterozygous *sp*/+ plants in the case of the C81 crosses. Data are available on this point. These are as follows:

	Seeds planted	Plants obtained and examined	Loss	% loss
P39 crosses	580	515	65	11.2
C81 crosses	512	351	161	31.4
			Difference =	20.2

There was a greater loss from the seed to the mature plant stage for the C81 crosses. If the 20 percent difference in loss is due to elimination of *sp*/+ plants then this amount should be added to the *sp*/+ plants already found for the C81 crosses. Adding this gives 295 *sp*/+ in a total of 453 or 65.1 percent. This leaves 34.9 percent ++ which represent crossovers only. Multiplying this by 16.4, the original "crossover+survival class" gives a corrected crossover value for the C81 crosses of 5.7 which is in agreement with that of the P39 crosses, 5.2 percent. Of course we cannot be sure the additional 20 percent loss for the C81 crosses is all due to elimination of *sp*/+ plants. The fact that *sp*/+ plants tend to be weaker than normal sibs supports the supposition that this is so. At any rate, something has caused the elimination of 31.4 percent of the zygotes between the seed and mature plant stage in the C81 crosses, whereas in the P39 crosses the loss was only 11.2 percent. It seems that the C81 inbred which eliminates more

of the *sp* male gametes than P39 also has a more deleterious effect on zygotes which are heterozygous for *sp*.

In addition to the variable functioning of *sp* pollen grains on different inbreds, and the functioning of *sp* male gametes when screened to remove competition of normal grains, we would expect an increased functioning of *sp* grains if competition were reduced by limiting the pollen so that part of the silks received only one functional grain. We have made no experiments on this point, but data secured from other experiments show that this may occur. Of the 20 ears obtained in 1926 from the backcross $su \times sp+/+su$ (89.9 percent sugary) approximately half of the ears were partly filled, some of them undoubtedly so because of insufficient pollen. These ears were arbitrarily divided into two groups, one with less than 200 seeds, the other with more than 200. There were 11 ears in the first group with an average of 127 seeds per ear; nine in the second group with an average of 424 seeds. The percentage of *Su* seeds (crossovers plus *sp* survivals) in the first group was 15.2; in the second, 8.2. The difference is highly significant ($P<.01$). Since the two groups represent random samples of the population in other respects, the differences cannot be attributed to variations in crossing over, and are undoubtedly due to differences in competition between *Sp* and *sp* grains resulting from variations in the amount of pollen available on the styles.

Quite different results were secured in 1930 when a homogeneous population of 55 ears with 94.1 percent of *su* seeds was divided the same way as those in 1926 into two arbitrary classes with more and less than 200 seeds each. In the first group there were 29 ears, in the latter 26. Each group of ears gave an average crossover percent of 5.9. The results of the two tests indicate that competition for limited pollen is undoubtedly a factor in some cases, and not in others.

A third source of variation should be sought in modifying factors which affect the expression of the *sp* gene. Some indication that this occurs is found in the fact that in some samples of pollen from *sp*/+ plants, the small grains can be definitely distinguished from normal grains; in other samples there is an overlapping of the two classes. Yet no correlation between this variation and that in the male gametic ratios has been found, nor is there any correlation between male gametic ratios in two successive generations. The differences are probably due primarily to variations in functioning of *sp* grains rather than to differences in crossing over.

That the variations in "crossing over" are really differences in *sp* pollen survival rather than differences in crossing over is shown by correlating the recombination percent in parent and offspring. If due to a difference in recombination we would expect such differences to be transmitted. The correlation of the crossover percents in parent and offspring is $-.28 \pm .091$

showing no positive correlation and perhaps a slight negative one. The negative correlation is just barely significant.

Effect of sp upon the female gametophyte

The factor *sp* is transmitted largely through the ovules, but there is some lethality of *sp* megaspores. Otherwise, ratios higher than 50 percent *su* could not be obtained for self-pollinations or backcrosses. The figures for these pollinations are 61 and 62 percents based on 143 thousand and 36 thousand kernels respectively. The elimination of *sp* megaspores is also indicated by missing places on heterozygous ears and by the reduced number of kernels compared with *Sp Sp* ears. A comparison of the number of seeds on 61 ++ ears and 237 *sp*/+ ears showed that the *sp*/+ ears had on the average 67.8 percent of the number of kernels on the former ears.

Since there is a semi-lethal action of the *sp* gene upon the megaspores the question arises as to what percent of the *sp* ovules survive and are functional. We can measure directly the percentage of *sp* ovules which accomplish fertilization and produce mature plants. In previously reported three-point tests with *sp su* and *Tu* (EMERSON, BEADLE and FRASER, 1935) 801 plants were grown of which 497 were normal and 304 were heterozygous for *sp*. If *Sp* and *sp* ovules had originally occurred in equal numbers the 304 *Sp sp* plants represent survival for approximately 497 ovules. This is a survival of 61.2 percent.

In the three-point test with *la*, *sp* and *su* reported later in this paper 1898 plants were grown of which 496 were heterozygous for *sp*. Calculated in the same way this represents a survival of 35.4 percent. The weighted average for the two populations is 42.1 percent, which is somewhat less than the 56 percent survival of *sp2* reported by RHOADES and RHOADES 1939.

Data already presented indicate that there is sometimes an elimination of heterozygotes between the mature seed stage and the mature plant stage. We can calculate the survival of *sp* through the megaspores to the mature seed stage by the following formula where s is percentage of survival, p = percentage of non-crossovers and m = percentage of functional *su* gametes among the ovules of $sp+/+su$ plants or the percentage of functional *Su* ovules in $sp\ su/++$ plants.

$$s = \frac{100(p-m)}{(p+m)-100}$$

If we assume that crossing over between *su* and *sp* is relatively constant at five percent[2] it is found that survival of *sp* through the megaspores to the mature seed stage varies from 0 to 100 percent in different progenies.

[2] Slight differences in crossing over have no appreciable effect upon calculated survival. The results are quite similar if the percentage of crossing over is computed at three percent, the minimum figure, or six percent the maximum.

The average survival in *sp*+/+*su* plants (61.9 percent *su*) is 58.2 percent; in *sp su*/++ plants (37.6 percent *su*) 56.8 percent.

The populations used in the two three-point linkage tests mentioned above permit a direct comparison of survival to the mature seed stage and survival to the mature plant stage. In the three-point test with *Tu* crossing over between *sp* and *su* was 5.8 percent and the percentage of sugary seeds in the backcrossed ears was 61.6 percent. The calculated survival of *sp* to the mature seed stage is 58.4 percent. This is slightly lower than the actual survival to the mature plant stage, 61.2 percent, and indicates that in this particular population there has been no elimination of heterozygotes after the mature seed stage. In the three-point test with *la* the crossing over between *sp* and *su* was 5.0 percent and the percentage of sugary seeds on the backcrossed ears was 36.7 percent. The calculated survival to the mature seed stage is 54.4 percent, which is considerably higher than the actual survival to the mature plant stage, 35.4 percent, and suggests an elimination of heterozygotes. Thus the data not only show extreme variations of survival of *sp* through the megaspores, but support the suggestion already made, that elimination of heterozygotes varies with the stocks used as parents.

The percent of *su* seeds when plants of the constitution *sp*/+ are selfed shows a decided correlation between one generation and the next. The correlation coefficient was $+.40 \pm .04$. This is highly significant. In Texas a similar comparison of six parents and progenies gave a correlation coefficient of $+.67 \pm .23$. Both of these comparisons show that there is a decided tendency for ears that produced a high percentage of *su* seeds one year to give a high percentage the next. Since any percent of *su* seeds in excess of 50 is caused by ovule elimination of *sp* ovules it is evident the degree of ovule elimination is transmitted.

Substitution of megaspores

In maize as well as in many other plants, the embryo sac develops from only one of the four megaspores resulting from the two divisions of the megaspore mother cell. The other three are lost. In several instances where peculiar genetic results have been encountered, it has been suggested that substitution of megaspores may have occurred; that when the megaspore which usually persists, receives a combination of genes with a lethal effect, it may be replaced by a viable sister megaspore. This has been shown by RENNER (1921) to occur in Oenothera and is known as the "Renner effect." Maize is ideal material for investigating this phenomenon because the orderly arrangement of the seeds renders any lethal effect immediately visible.

That the substitution of megaspores in *sp*/+ stocks, if it occurs at all, is not complete, is at once apparent from the fact that ears from hetero-

zygous plants are seldom well-filled and usually exhibit many missing kernels. However, if there is any substitution whatever the number of gaps should be smaller than the number expected to occur on the basis of the calculated survival of *sp* through the megaspores.

In 1926 we compared 29 ears from normal *Sp Sp* plants with 77 ears from *Sp sp* plants. The former had an average of 263 seeds per ear, the latter 179 seeds. Thus, the high-sugary ears had only 68.1 percent as many seeds as normal ears from the same progenies. The average survival of *sp* in 1926 was calculated as 47.4 percent. This means that high-sugary ears in 1926 would be expected to have, on the average, 73.7 percent, $50+(47.4/2)$, as many seeds as normal ears. The difference between this figure and the one actually obtained, 68.1 percent, is easily accounted for as the result of sampling errors. In any case, the difference is in the wrong direction to indicate substitution of megaspores.

In 1930 we compared 160 high-sugary ears with 32 normal ears. The former had an average of 238 seeds per ear, the latter 377. The lower figure is 63.1 percent of the higher. Survival of *sp* in the megaspores in 1930 was computed at 31.0 percent. On this basis ears from *Sp sp* plants would be expected to bear 65.5 percent as many seeds as ears from normal plants. Again the difference is in the wrong direction to suggest substitution of megaspores. The averages for the two years are 69.6 percent calculated and 65.6 percent actually found. That the number of seeds found on ears from *Sp sp* plants is lower in both cases than the number calculated on the basis of survival of *sp* is probably due to the fact that plants heterozygous for *sp* are slightly weaker than normal plants and hence probably bear slightly smaller ears. That the observed and calculated figures agree as closely as they do indicates not only that no substitution of megaspores has occurred, but also that the data on survival of *sp* in the megaspores are reasonably accurate.

Effects of sp upon the sporophyte

Effect upon the heterozygote

That *sp* has a deleterious effect upon the heterozygote has already been suggested by the fact that its survival through the megaspores to the mature seed stage is sometimes greater than survival to the mature plant stage of the succeeding generation. Additional observations support this suggestion.

Plants heterozygous for *sp* are slightly less vigorous and later in blooming than their normal sibs. It is not always possible to demonstrate this fact statistically because of other variables, but it has been observed repeatedly especially in certain progenies. In one season at least (1926), the difference in height of stalk, 2.9 inches, was statistically significant ($P<.03$).

Effect upon the homozygote *sp sp*

Since *sp* survives in part of the megaspores, and *sp* pollen can function, it should be possible to obtain seeds homozygous for *sp*, and if these are viable to determine the effect of *sp*/*sp* upon the plant. Pollen screening experiments in both Texas and Connecticut have altered the *Su*:*su* ratio, and have resulted in a considerable functioning of *sp* pollen. So far no homozygous *sp*/*sp* plant has been found either in ordinary populations or in cases where screened pollen was applied to heterozygous *sp* plants. In one experiment where it was determined that at least 28.7 percent of the functional ovules were *sp* and at least 73.5 percent of the functional pollen grains were *sp* no homozygous plants were obtained in a population of 78 plants derived from 176 seeds. Seedling mortality was high even under ideal conditions which suggests that the homozygous combinations which must have occurred from this pollination were eliminated as seed or seedling lethals.

THE EFFECTS OF *lo*

Effect upon the female gametophyte

The chief effect of *lo* is upon the female gametophyte. The majority of ovules receiving *lo* are lethal, and ears from heterozygous plants are always poorly filled, resembling ears from "semi-sterile" plants in which reciprocal translocations are involved. There is however some survival through the megaspores as was discussed in a previous section. One such test where *lo su*/+*su* ears were pollinated by *Su* gave 465 ++ ears and nine *lo*/+ ears or 1.9 percent of *lo*/+ ears. Since this represents an unselected sample it seems safe to say that about two percent of *lo* ovules can take place in fertilizaton.

Effect upon the male gametophyte

There is no visible effect of *lo* upon the male gametophyte, and pollen from *lo*/+ plants is quite normal in appearance. Furthermore, there is apparently no elimination of *lo* through the male gametophyte, in fact the contrary seems to be true. In the backcross *su*×*lo*+/+*su*, the percentage of + seeds was 51.5, while in the backcross *su*×*lo su*/++, the percentage of *su* seeds was 51.3. The total number of seeds in these two populations is 51,606, of which 26,541 or 51.4 percent, represents the class in which *lo* predominates. The deviation from the 50.0 percent expected in this class is $1.4 \pm .148$. The deviation is 9.5 times the probable error, and the odds against its chance occurrence are more than a billion to one.

This situation in which a gene is almost completely lethal to the ovules but advantageous to the male gametophytes receiving it, is unique. Perhaps a hormone-like action which stimulates the male gametophyte and retards the female gametophyte is involved.

Effect upon the sporophyte

We have never noticed any effect of *lo* upon the sporophyte. Plants of the constitution *lo*/+ seem to be as vigorous as those of the constitution +/+ and there is no noticeable difference in time of flowering.

No plants homozygous for *lo* have been obtained. It should be possible to obtain such individuals since about two percent of the functional ovules are *lo* and since the *lo* pollen functions normally. An attempt will be made to obtain homozygous *lo*/*lo* plants.

LINKAGE RELATIONS OF *sp* AND *lo*

Linkage relations of sp

In a previous section we have found the recombination value for *sp* and *su* to be approximately five percent.

TABLE 6

Three-point linkage tests with sp and lo.*

F_1 GENOTYPE	PARENTAL COMBINATIONS	RECOMBINATIONS REGION 1	REGION 2	REGIONS 1 AND 2	TOTAL
Ts5 + + / + *sp su*	738 —	— 50	10 —	— 4	802
		6.2	1.2	.5	
Ts5 sp + / + + *su*	— 367	77 —	— 45	24 —	513
		15.0	8.8	4.7	
Total	1105	127	55	28	1315
		9.7	4.2	2.1	
la + +†/ + *sp su*	781 439	781 14	41 16	2 35	1898
lo + + / + *su gl3*	1748	161	1048	52	3009
		5.4	34.8	1.7	

* For additional tests already published see EMERSON, BEADLE and FRASER 1935.

† Backcross for *su* and *sp* F_2 for *la*.

Various three-point tests with *sp su* and *Tu*, the results of which have already been published (EMERSON, BEADLE and FRASER 1935), show *sp* to be to the left of *su*. Several three-point tests with *Ts5 sp* and *su*, show *sp* to lie to the right of *Ts5* and to the left of *su*. The data are shown in table 6.

The three-point test for *la*, *sp* and *su* given in table 6 proved to be an F_2 for *la* and a backcross for *sp* and *su*. Evidently a +*su*/*la su* plant instead of the double recessive was used for a pollinator. The cross was then *la*++/+*sp su*×*la su*/+*su*. The crossover value for the three genes is as follows: *la* 7.5 *sp* 5.0 *su*. The recombination value for *la* and *su* was 13.2 percent. The crossover values for *la* and *sp*, also for *la* and *su* were calcu-

lated by the product method (IMMER 1930) using the formula ad/bc $=p+p^2/2-3p+p^2$ where p equals the crossover value. The crossover value for *sp* and *su*, 5.0 percent, was calculated directly since this was a backcross for both, and survival of *sp* in the pollinations not involved.

The only data which suggest that *sp* might be to the right of *su* are those showing the crossing over between *sp* and *gl3* to be 26 percent. Published data (EMERSON, BEADLE and FRASER 1935) show crossing over between *su* and *gl3* to be 34 percent, and the much lower crossing over between *sp* and *gl3* would suggest that *sp* lies between *su* and *gl3*. Since all three-point tests show that the contrary is true, this is another example of the variations involved in comparing crossover values from different stocks.

One additional linkage test with *sp* should be mentioned because of the peculiar ratios obtained. The gene *de16*, which causes defective seeds, is located on the fourth chromosome about 3.2 units to the right of *su*. Plants of the composition *sp* + +/+ *su de16* should, when selfed, be high-*su* and high-*de16* seed. In most ears, however, the *su* gene does not express itself in seeds homozygous for *de16*, and since *su* and *de16* are closely linked, there is actually a decided deficiency of visible *su* seeds. In a total population of 2609 seeds from 11 ears, 1430 or 54.8 percent of the seeds were *de16*, while 106 or 4.1 percent of the total were visibly *su*. Yet when pollen from one of these plants was applied to normal *su* plants the percentage of *su* seeds produced was 90.6. A similar situation has been described in which the gametic lethal *lo* is involved.

Among those 11 ears was one in which the + and *su* endosperms in the defective seeds were distinguishable. The four classes of seeds occurred in the following numbers: ++, 117; +*su*, 21; *de16* +, 15; and *de16 su*, 67. Since 40.0 percent of all seeds are *su* and 37.3 percent are *de16*, the results suggest that *sp* has had a greater effect upon *su* than upon *de16* and hence must be closer to *su* than to *de16*. Thus these data, though not critical, are in agreement with other three-point tests which place *sp* to the left of *su*.

Linkage relations of lo

It has already been shown that the percentage of recombinations for *lo* and *su* in the ovules is 1.6 percent. Likewise in published data (EMERSON, BEADLE and FRASER 1935) the recombination percent in the pollen for the repulsion backcross was 1.4 percent. This is very close to the value for ovules. Since the publication of that figure a few additional progenies have been summarized which may alter slightly the recombination percent observed from the pollen. All data are summarized in table 7.

These values are variable especially in the coupling phase. Not much reliance is placed on the figures for this phase since the figures for the most part represent compilations of small progenies.

TABLE 7

Recombination values from backcrosses of lo and su in pollen.

	REPULSION PHASE				TOTAL	RECOMBINATIONS	
	++	+ *lo*	*su* +	*su lo*		NO.	%
1	1	85	80	0	166	1	0.63
2*	1	444	78	2	525	3	0.57
3*	9	190	185	1	385	10	2.6
4	0	92	124	18	234	18	7.7
5	11	265	288	7	571	18	3.2
6	5	160			165	5	3.1
7	23	805	776	24	1628	47	2.9
Total	50	2041	1531	52	3674	102	2.8
			COUPLING PHASE				
8	34	2			36	2	5.6
9	197	19	19	179	412	38	9.2
10	52	15	9	50	126	24	19.0
11	44	0	0	45	89	0	0
Total	327	36	28	274	665	64	9.7

* The totals of 2 and 3 give an average of 1.4 percent, the figure published by EMERSON, BEADLE and FRASER 1935.

In the repulsion phase, the average of all years' trials before 1939 is 2.7 percent. This is higher than the values found from the ovules after correcting for *lo* ovule survival. The values obtained for different progenies vary considerably, from .57 percent to 7.7 percent for one progeny of 234 plants where all the recombinations were of the *su lo* class and there were none of the ++ type. Omission of this progeny from the calculations brings the average figure to 2.4 percent. In four of the progenies, totaling 681 plants, in the repulsion phase the crossover value was less then one percent. It is evident there is considerable variation in the crossover values from the pollen. Part of this might be caused by mistakes in classification, as all *lo*/+ plants were classified entirely by the semi-sterile appearance of the ear. Although on the whole this is a reliable criterion, in the case of ears poorly filled for other reasons there may have been a few mistakes. Also, crossovers of the constitution ++ could have arisen from a contamination in the original pollination. In the 1939 data it is doubtful if this could have been a factor since extreme care was taken to guard against contaminations in the original pollinations. The fact that both crossover classes are numerically so nearly alike leads us to believe that errors in classification were negligible in these progenies.

The conclusions regarding the percentage of recombination between *lo* and *su* are that in the pollen the percent for the repulsion phase varies from one to three with an average of 2.8. The recombination value in the ovules for both coupling and repulsion phases was 1.6. There may be a dif-

ference in the amount of crossing over in microsporocytes and megasporocytes. Since the variation of the crossover values is from 1.6 to 2.8, no serious error will be encountered if any value between those figures is taken as the recombination value for *lo* and *su*. If we take the mid-way point for these two figures we get a value of 2.2 percent (which is the average value published in 1935 by EMERSON, BEADLE and FRASER). The crossover value of 2.2 will be used for *lo* and *su* in calculating linkage with other factors on chromosome 4.

Recombination value of *lo* and *Ts5* is eight percent, and there are two percent of recombinations between *lo* and *de16* (EMERSON, BEADLE and FRASER 1935). Other linkages of *lo* on chromosome 4 are shown in table 8.

TABLE 8

Crossover values of sp and lo with gl3.

GENES X AND Y	LINKAGE PHASE	XY	Xy	TOTAL	RECOMBINATIONS NO.	%
Lo Gl3	RB	1722	2924	4646	1722	37
Sp Gl3	RB	1210	3530	4740	1210	26

This shows 37 percent of recombinations between *lo* and *gl3*. This would tend to place *lo* to the left of *su*, since *gl3* and *su* show 34 percent of recombinations. However, where two factors are as far apart as 34 units they are not very useful in locating a third gene which is close to one of them. The data with *de16* are more conclusive. This lethal shows 3.2 percent of recombinations with *su* and the value for *lo* and *su* is 2.2 percent. Hence if *lo* were on the left of *su* we should expect about five percent of recombinations between *lo* and *de16*. Actually there was two percent of crossing over between *de16* and *lo* which would be expected if *lo* were to the right of *su*. These data agree with the three-point test between *sp*, *su* and *lo* placing *lo* to the right of *su*. One three-point test for *lo*, *su* and *gl3* was summarized in table 6. The crossing over between *lo* and *su* is 7.1 percent; between *su* and *gl3*, 36.5 percent; between *lo* and *gl3*, 40.2 percent. These data were obtained from crosses in which *lo* survival in the ovules could have been a disturbing element. It is impossible now to correct for that factor although the 7.1 percent of crossovers between *su* and *lo* indicate a survival of *lo* ovules. Even if corrected it would not alter the relative positions of the three factors calculated from these data. This three-point test tends to place *lo* to the left of *su*, in direct conflict to the three-point data of *sp*, *su* and *lo* and emphasizes the variations found in this study.

Combinations of sp, lo and su

The combination of sp $++/+$ *su lo* gives the unusual result described in the introduction and shown graphically in figure 3. Actual ratios are

found in table 2. At the time *lo* was discovered it was not known on which side of *su* it was located. If on the same side it would not be improbable that the two were alleles of the same gene. However a simple test rules this out and also gives valuable data on the location of *lo* in relation to *su*. The pollen from a plant, *sp* ++/+ *su lo*, was applied to *su* silks. If *lo* and *sp* were alleles of the same gene then the progenies should either segregate *lo* or *sp*, and no normal ratios should be found. The cross of *su* by *sp* ++/+ *su lo* gave 33 selfed ears. Thirty-one segretated in a normal 3:1 ratio for *su* and two segregated for *sp*. No *lo*/+ ears were found. The occurrence of the 3:1 ratios proves that *sp* and *lo* are not allelic. These ratios also tend to show that *lo* and *sp* are on opposite sides of *su*. If on the same side, the composition would be *sp* ++/+ *lo su* with the crossover values *sp* 2.8 *su* 2.2 *lo* since *sp* is five units from *su*, and *lo* and *su* are 2.2 units apart. Hence we should expect the crossovers at region 2 to be almost equal to those at region 1, 2.8:2.2 percents respectively. The functional male gametes should be +++ (56 percent) and + *lo* + (44 percent). Only *Su* seeds were planted, so *su* gametes need not be considered. A similar comparison of *su* seeds from the cross *su*×*sp su*+/++ *lo* gave similar results. Both sets of data are summarized below.

	Crossovers Region 1	Crossovers Region 2
su×*sp*++/+*lo su* (*Su* seed)	31	0
su×*sp*+*su*/+*lo*+ (*su* seed)	200	12
Total	231	12
Expected (56%:44%)	136	107

This deviation is highly significant (P <.01). The crossovers at region 2 were far too few. If *sp* and *lo* are on opposite sides of *su* then we would expect very few of the second class, double crossovers only. Although not conclusive, these data strongly suggest that *sp* and *lo* are on opposite sides of *su*.

In 1939 a three-point test was conducted for *sp*, *su*, and *lo*. The 212 plants just described were taken from this three-point test. Complete results are presented in table 9. In planting this test it was not possible to grow all the seed available and no effort was made to grow the same relative amounts of *Su* and *su* seed that occurred in the original pollinations. We were more interested in the plants produced by the *su* seeds so relatively larger amounts of the *su* seed were planted. In calculation it was necessary to correct for the *su* seed grown, reducing the *su* seed to the same proportion found in the original crosses. This correction factor is given in table 9.

Also there was some elimination of *sp* male gametes, more in the C81 crosses than in the P39 hybrids. In order not to discriminate against the *sp*

classes it was necessary to increase the individuals in these classes. This was done by dividing the *sp* class found by the percentage of *sp* individuals found, 86.8 percent in the P39 crosses, and 56.4 percent in the C81 crosses.

The use of this correction factor may be questioned but we believe by using it the recombination values are much more accurate than would have been obtained had no correction factor been used. It would be desirable, of course, to have additional data from experiments where no correction factor was necessary.

TABLE 9

Three-point test for sp, su and lo. F_1 genotype=sp su +/++ lo.

	P		R 1		R 2		R 1 and 2		
	sp *su* +	+ + *lo*	*sp* + *lo*	+ *su* +	*sp* *su* *lo*	+ + +	*sp* + +	+ *su* *lo*	TOTAL
P39×1174-4, 1176-5, 1176-10	259	272	4	31	7	4	4	4	
P39 corrected for *su* seed planted.*	138	272	4	16.5	3.7	4	4	2.1	
P39×1176-8	65	99	1	11	1	0	0	3	
P39 corrected for *su* seed†	42	99	1	7.1	.6	0	0	1.9	
Total P39 crosses corrected for *su*	180	371	5	23.6	4.3	4	4	4	
Total corrected for *sp*=*sp* class/86.8	207	371	5.8	23.6	5.0	4	4.6	4	
	578		29.4		9.0		8.6		625.0
			4.7%		1.4%		1.4%		
C81 crosses	210	355	7	15.8	4	9	9	5	
C81 corrected for *su*¶	36.1	355	7	27.2	.7	9	9	.9	
C81 corrected for *sp*=*sp* class/56.4	64.0	355	12.4	27.2	1.2	9	16.0	.9	
	419		39.6		10.2		16.9		485.7
			8.2%		2.1%		3.5%		
Total	997		69.0		19.2		25.5		1110.7
			(6.2%)		(1.7%)		(2.3%)		

* Seeds in ratio of 2714 *Su*:1744 *su* planted in ratio of 120:145
Correct number of *su* should have been 120:X::2714:1744=77.1 seeds.
77.1/145=53.2 percent *su* class×53.2 percent=corrected *su* class.

† *Su*:*su* planted=145:145

¶ Seeds in ratio of 7089:1478 planted in ratio of 120:145

A comparison of the recombination values in table 9 shows 6.2 percent for *sp* and *su;* 1.7 percent for *su* and *lo* and 2.3 percent of double crossovers, more than the crossover percent for region 2. This suggests that *sp* and *lo* are on the same side of *su*. Either way the experiment is calculated, the percent of double crossovers is much too high and suggests either faulty classification or that some other factors such as pollen contamination or hetero-fertilization may have tended to increase the individuals in the double crossover class. We have encountered several cases of apparent hetero-fertilization during the course of this investigation. If any of the seeds in the double crossover class were cases of hetero-fertilization they would belong in the parental rather than the double crossover class.

From all the data we would be forced to conclude that it is not possible to tell whether *sp* and *lo* are on the same or opposite sides of *su*. However the most critical part of the data, as well as previous data, strongly suggest opposite sides, as was discussed previously.

CYTOLOGY OF *sp*/+ AND *lo*/+ PLANTS

In regard to the unusual behavior of the *sp* and *lo* genes, it is important to know if any cytological disturbance is present. In a preliminary report (SINGLETON 1932) it was stated there were "no conspicuous irregularities of chromosomes at the reduction division." This referred to examination of the chromosomes in mid-prophase of meiosis. Since that first examination of the pachytene chromosomes, STADLER (1933) has demonstrated a case of small pollen in maize associated with a deficiency in chromosome 10. STADLER'S small pollen behaved genetically similar to *sp*. Since these cases are so much alike, reexamination of chromosome 4 in a number of plants known to be segregating for *sp* was undertaken. A greenhouse progeny in Connecticut was examined cytologically in 1939. Four plants, heterozygous for *sp*, later verified by pollen examinations, were examined at mid-prophase of the first meiotic division. No deficiency was observed in any plant, and pairing of all chromosomes was normal. The factors *sp*, *lo* and *su* are on chromosome 4 which was studied particularly for any evidence of a chromosomal alteration. Pachytene chromosomes have also been repeatedly examined in Texas in plants heterozygous for *sp*, and likewise no deficiency was observed. Plants heterozygous for *sp2* on chromosome 10 also showed no visible deficiency. (RHOADES and RHOADES 1939.)

Two plants, heterozygous for *lo*, were examined in Connecticut in 1939. No deficiencies were observed in any of the chromosomes 4 examined, and all other chromosomes were apparently also normal (figure 5).

DISCUSSION

There are now on record many occurrences of maize plants segregating small pollen, some spontaneous, others induced by X-rays or by ultraviolet radiation. In some instances these may be associated with a visible chromosomal deficiency (STADLER 1933) but in most of the cases so far observed no chromosomal irregularities can be detected. In most segregations for small pollen, the smaller grains are comparatively unable to effect fertilization in competition with the normal larger grains. This condition has been observed so often that it is now regarded as of general occurrence that the small pollen grains are not capable of competing with the larger grains.

In our study of *sp* several observations have shown a considerable proportion of *sp* grains that accomplished fertilization in competition with

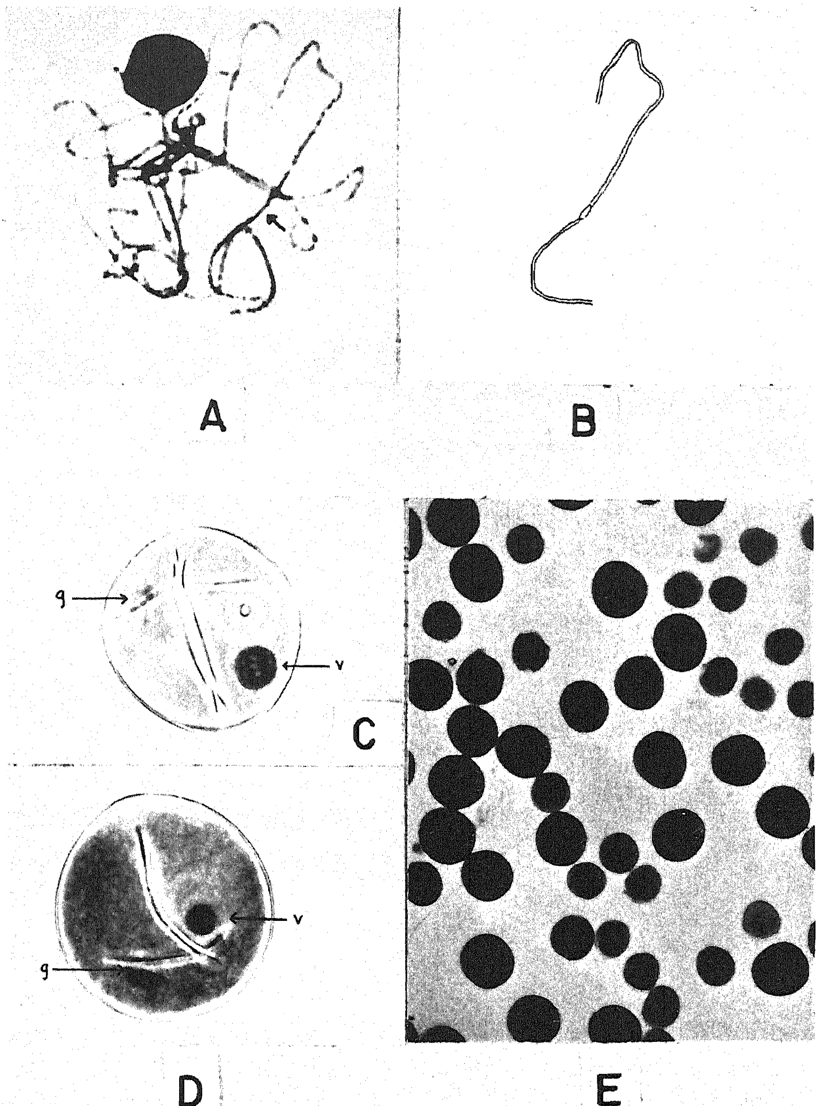

FIGURE 5.—A, pachytene preparation from $sp+/+su$ plant showing chromosome 4. Arrow points to centromere. No evidence of lack of pairing or heterozygous deficiency. B, drawing of chromosome 4 in A. C, small pollen-1 microspore before division of generative cell. v=nucleolus of vegetative nucleus, g=generative nucleus. Note vacuolated condition of nuceolus of vegetative nucleus. D represents a normal microspore from same plant as C which was segregating *sp*. Note larger size of microspore, denser cytoplasm and smaller but more compact nucleolus of the vegetative nucleus. D is more completely filled with starch at this stage. E shows pollen from $sp+/+su$ plant stained with iodine. Smaller grains in most cases well filled with starch.

normal; in pollination from one plant in 1938 there was no competitive effect whatever. Also, the stock to which the pollen is applied can make considerable difference in the amount of small pollen functioning.

So far as we are aware, no gene mutations have been found responsible for any gametic lethals in maize. The *sp* reported by MANGELSDORF for the first time in 1931, was the first case of a partial gametic lethal to be observed that was not associated with a visible chromosome abnormality. It is possible that *sp* may be caused by an undetectably short deficiency, but as stated before there is no visible alteration of chromosome 4 in stocks segregating for *sp*. If the abnormality is undetectable, we have no way of knowing whether it has a cytological basis or whether it is in the nature of a gene mutation.

In some ways it behaves like a gene mutation: it is transmissible through the ovules, in some cases with very little or no ovule elimination; in some instances, when pollen from a heterozygous plant is used, the *sp* grains function almost as well as the normal grains. Usually, however, there is almost complete elimination of *sp* grains, the lethal action being complete from a functional standpoint. In linkage relations with characters on chromosome 4, *sp* also behaves like an ordinary gene.

In one important respect the *sp* factor definitely behaves as a deficiency: plants heterozygous for *sp* are quite often smaller than normal sibs. This was especially true when the stock was first grown in 1926. Also one progeny grown in 1927 showed several abnormally small plants. At various other times we have noted that plants heterozygous for *sp* were later in maturity and not as vigorous as sib plants. In this way the factor *sp* behaves more like a chromosomal deficiency than like an ordinary recessive gene. We know of no convincing evidence indicating that a recessive gene in the heterozygous condition causes the plant to be noticeably smaller than the homozygous dominant. All normal recessive genes tested have given just as vigorous plants in the heterozygous condition as do the normal sibs. MANGELSDORF (1928), KARPER (1930), and ROBERTSON (1932) have shown that recessive seed and seedling lethals in maize, sorghum, and barley, respectively, have no measurable effect upon the heterozygote. The *sp* factor seems to be a border line case, cytologically invisible, which acts both like a gene and a deficiency.

This condition is similar to the situation in Drosophila. SLIZYNSKA (1938) analyzed the salivary gland chromosomes of 14 genetic deficiencies in the white-facet region. These are all lethal when homozygous. The longest of the cytological deficiencies included 45 bands, five included one band and one was undetectable cytologically. This last case is like the *sp* condition. This deficiency in Drosophila was a zygotic lethal whereas *sp* is a functional gametic lethal, as well as a zygotic lethal.

SLIZYNSKI (1938) studied 19 spontaneous lethals and 13 lethals induced by X-rays. Of the spontaneous lethals, nine were associated with a cytological deficiency and four of the 13 induced cases were visible cytologically. In more than half of all the cases studied, however, no deficiency in the salivary chromosomes was visible. The reverse condition is shown by DEMEREC and HOOVER (1936). They found a visible cytological deficiency having no genetic effect. DEMEREC (1940) has also made quite an extensive study of X-ray induced and spontaneous Notches in Drosophila. In 27 X-ray induced Notches, three had the full complement of bands, in seven cases one band was missing, in four cases two to five bands were missing and in 13 cases more than six bands were missing. In 10 spontaneous Notches there was one with no bands missing, and nine with one to more than six bands deficient. Thus, lethals and detectable chromosomal deficiencies, although sometimes associated, are not necessarily so.

The *sp* condition in most cases is a functional lethal although the gametes are produced, and the lethality is not necessarily complete. The *lo* factor, however, is a true gametic lethal, the action taking place early in the development of the female gametophyte. Ovule elimination is almost complete for *lo* ovules. In no cases have we found more than a very small percent of the *lo* ovules functioning. This is the only gene of its kind in maize, with its lethal action confined exclusively to the ovules. All other conditions, so far described, that affect the gametes have a more pronounced effect on the male than on the female gamete. Chromosomal disturbances, deficiencies and duplications, have this affect. The *lo* factor however, exercises its lethal affect solely on the ovules. If there is any affect on the male gametes it is one of stimulation since the ratios when pollen from *lo*/+ plants is applied to normal stocks there is an excess functioning of *lo* gametes. Nothing is known of the mechanism whereby *lo* pollen grains are favored over the normal. There seems to be some selective action, making the *lo* gene act as a male influence.

SUMMARY

1. The gene *sp* (small pollen) causes pollen grains to be reduced in size. In plants heterozygous for *sp* two sizes of pollen grains are produced in approximately equal numbers.

2. Pollen grains carrying the *sp* gene usually do not function in competition with normal grains, hence the gene is usually not transmitted through the pollen. When competition between + and *sp* grains is reduced or eliminated as a result of sifting the pollen or because of a sparsity of pollen on the styles, the *sp* pollen grains are capable of accomplishing fertilization. Pollen grains carrying the *sp* gene also regularly function successfully on the styles of certain maize stocks.

3. The *sp* gene is transmitted through part, but not all, of the female gametes; survival of *sp* through the megaspores varies from 0 to 100 percent. The average survival is 42.1 percent to the mature plant stage of the succeeding generation.

4. Plants heterozygous for *sp* are usually slightly weaker than their normal sibs. Heterozygous seeds are slightly smaller and frequently germless.

5. The gene *sp* is a seed or seedling lethal in the homozygous condition; no mature homozygous plants have ever been obtained.

6. The *sp* gene is linked with the *su* locus with approximately five percent of crossing over. In the repulsion phase this results in high-*su* ratios, selfed ears producing 61 percent of *su* seeds and backcrosses by and on *su* producing 62 and 93.9 percent of *su* seeds, respectively. In the coupling phase the ears are low-*su* and the corresponding percentages are 3.2, 38 and 9.7, respectively.

7. Although *sp* has many of the characteristics of a haplo-viable deficiency, cytological studies have failed to disclose such a chromosomal condition.

8. The gene *lo*, which appeared in a stock segregating for *sp*, is a gametic lethal with unique effects. It eliminates practically all the megaspores which receive it but has no effect, or a slightly stimulating effect, upon the male gametophyte.

9. The *lo* gene is also linked with the *su* locus, crossing over being approximately two percent between *lo* and *su*. In the repulsion phase the percents of *su* seeds resulting from selfing and backcrossing by and on *su* are 47.1, 95.2, and 48.5, respectively. In the coupling phase the corresponding percents are 1.8, 2.4, and 51.3, respectively.

10. The *lo* gene is not allelic to *sp*. Plants heterozygous for *sp*, *lo*, and *su* produce 5.2 percent of sugary seeds when selfed, 8.5 percent when backcrossed by *su*, and 91.6 percent when backcrossed on *su*.

11. Various linkage tests with other genes on chromosome 4 show that the order of the genes is probably *Ts5 la sp su lo de16 Tu gl3*.

We are indebted to MISS FRANCES CLARK and DR. R. G. REEVES for preparation and examination of part of the pachytene material. Also, MISS CLARK found the differences observed in the microspores between *sp* and +.

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THE EFFECT OF AN EARLY LETHAL (t^0) IN THE HOUSE MOUSE

S. GLUECKSOHN-SCHOENHEIMER[1]
Columbia University, New York

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INTRODUCTION

THREE strains of mice in which mutations have brought about malformations of the tail have proved to be of special interest for developmental studies. These mutations affect very early stages of embryonic life, and have thus made possible the experimental analysis of some early developmental steps in mammals which had not before been open to attack, because of experimental difficulties. The three factors involved are probably alleles (they do not show any crossing over), and their effects on early development are not the same. Thus, they offer good material for a study of the relationship of the developmental effects of three unilocal factors.

One of the three strains of mice concerned is short-tailed, and two are tailless. The development of the short-tailed heterozygote ($T+$) and the homozygote (TT) which dies on the 11th day of intrauterine life, have been described by CHESLEY (1935). He found the abnormalities in both the heterozygote and the homozygote to center around a deficiency of the notochord. This structure is nearly completely absent in the homozygote TT and is missing in that part of the tail of the heterozygote $T+$ which is to be constricted and resorbed in the latter half of embryonic life.

The genetical analysis of tailless line A, discovered by DOBROVOLSKAIA-ZAVADSKAIA and KOBOZIEFF (1927) proved it to be heterozygous for T and t^0 (CHESLEY and DUNN 1936). Matings of Tt^0 by Tt^0 produce only tailless progeny at birth; actually, three types of embryos are formed of which only Tt^0 (tailless) is viable while TT dies on the 11th day of pregnancy, and t^0t^0 dies shortly after implantation. The maintenance of the line as a balanced lethal system is due to the absence of crossing over between the two lethal factors T and t^0. The second tailless line (29) is heterozygous for T and t^1 (DUNN 1937) and behaves similarly to Line A, breeding true for taillessness and yielding three types of embryos, TT, lethal on the 11th day, Tt^1, tailless and viable, t^1t^1, lethal before implantation (GLUECKSOHN-SCHOENHEIMER 1938b).

The study of the development of the tailless embryos in lines A and 29 has shown that failure of the notochord in the tail is probably the chief cause for the resorption of the tail in later stages.

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A study of the effects of t^0t^0 on the embryo seemed to be of special interest, first because it produces its effect in a very early embryonic stage, immediately after implantation of the embryo, and second, because the manifestation of other mutations at this locus (TT, $T+$, Tt^0) appears to involve early changes in some of the primary structures such as the notochord and the neural tube, and it is thus important to determine whether the earlier effect of another combination of factors at this locus (t^0t^0) changes the development of structures antecedent to the notochord and neural tube.

The following investigation was undertaken as a continuation of CHESLEY'S studies on the t^0 homozygote. From examinations of pregnant uteri from tailless by tailless matings, on the sixth, seventh and eighth day of pregnancy, CHESLEY assumed that resorption of the abnormal type began on the sixth day and was completed by the eighth day. In the present study, it was possible to find the lethal embryos before their death and thus to study their morphological characters and the exact time of death.

The author wishes to thank DR. L. C. DUNN for the suggestion of the problem and for his aid and advice during the progress of the work.

MATERIAL AND METHODS

All animals used in these experiments were from the first to fifth backcross of DR. ZAVADSKAIA'S original stock (A line) to an inbred stock of Bagg albinos. The embryos were timed by the vaginal plug method. Male and females were left together for two to six hours and the females then were examined for vaginal plugs. The age of the embryos was thus known within ± 1 to ± 3 hours. The embryos were obtained from the mother as described by GLUECKSOHN-SCHOENHEIMER 1938a. Some of the embryonic capsules were dissected for diagnosis of possible gross malformations (40 embryos from four litters), but the rest of them (239 from 25 litters) were preserved and studied histologically. The embryos were fixed in Bouin's fluid, imbedded in paraffin and sectioned at 8 μ. The embryonic capsules were sectioned longitudinally. The orientation of the embryo within the capsule is not constant enough to permit of the determination of sagittal and frontal sections at imbedding. It is, however, possible to distinguish between the long and short axis of the embryonic capsule; as the embryo itself is usually orientated in mesometral-antimesometral direction (that is, along the long axis), it is thus possible to distinguish between cross and longitudinal sections. But it has to be left to chance whether sagittal or frontal sections are obtained.

FREQUENCY OF ABNORMAL EMBRYOS

Table 1 presents a summary of all dissections. Matings of $+t^0$ females by $+t^0$ males or of $+t^0$ females by Tt^0 males were used. Twenty-nine pregnant

females were dissected on various days after fertilization and a total of 279 embryos was obtained. Of these, 32 were dissected before the time when a diagnosis of typical t^0t^0 is possible. Of the remaining 247 embryos, 162 were normal and 85 abnormal. The ratio of normal to abnormal shows a significant departure from the expected 3:1 ratio, an excess of abnormals being found (35.1 percent instead of the expected 25 percent). This agrees with the results of CHESLEY and DUNN (1936) who in the genetic analysis of the A line observed an excess of offspring with t^0 wherever t^0 is introduced from the male. Their dissections of tailless females pregnant by tailless males revealed an excess of t^0t^0 offspring. From dissections of matings of $+t^0$ by $+t^0$ they reported 35.6 percent of the lethal type t^0t^0, instead of the expected 25 percent. The same phenomenon was observed in another tailless strain, line 29, carrying T and another allele, t^1. In this line also there was always an excess of offspring carrying t^1 wherever t^1 was introduced from the male, as discussed by DUNN and GLUECKSOHN-SCHOENHEIMER (1939).

TABLE 1

AGE DAYS	HOURS		DAYS	HOURS	NO. AND TYPE OF LITTERS	TOTAL	NORMAL	ABNORMAL	%
4	23	–	5	3	$3(+t^0\times+t^0)$	32	29	3	9.4
5	5	–	5	22	$3(+t^0\times Tt^0)$	28	19	9 (1 atypical)	32.1
5	18	–	5	23	$4(+t^0\times+t^0)$	28	20	8	28.6
6	0	–	6	20	$8(+t^0\times+t^0)$	81	57	24 (4 atypical)	29.6
6	3				$1(+t^0\times Tt^0)$	9	6	3 (1 atypical)	33.3
7	2	–	7	3	$3(+t^0\times+t^0)$	31	18	13 (2 atypical)	41.9
7	3	–	7	22	$3(+t^0\times Tt^0)$	34	21	13	38.2
8	0	–	8	3	$4(+t^0\times+t^0)$	36	21	15	41.7
Grand total					29	279	191	88	31.9
Total classified*					26	247	162	85	35.1

* Total embryos aged 5 days 5 hours to 8 days 3 hours; that is, those in which lethals could be distinguished.

The earliest litters examined in the present experiment were between the ages of four days and twenty-three hours and five days and three hours. During that time the difference between normals and lethals is not yet apparent; the few abnormals found probably resulted from accidental causes. From the age of about $5\frac{1}{4}$ days on, a group of embryos is histologically clearly different from the normals and can be classified easily. In frequency this group corresponds roughly to the proportion of homozygotes expected. The percentage of this type of abnormal embryos found from the beginning to the end of the sixth day is around 30 percent, both in matings of $+t^0$ by $+t^0$ and of $+t^0$ by Tt^0. At the age of six days the percentage of abnormal embryos also is around 30 percent while at the age of seven and

eight days it is found to be higher, namely around 40 percent. At that age the lethal embryos are dead and in the process of being resorbed; their classification is thus not based upon any distinct histological differences. In the group of "dead and resorbed" at the age of seven and eight days some embryos may have been included which genetically were not t^0t^0 but had died from accidental causes. Of the 88 abnormal embryos found in these dissections, eight were classified as atypically abnormal, that is, they did not show the characteristic features of the majority of abnormal embryos. It cannot be ascertained whether or not these embryos fall into the class of lethals. But even when they are subtracted from the total, the percentage of abnormals does not change significantly.

EARLY DEVELOPMENT OF THE NORMAL MOUSE EMBRYO

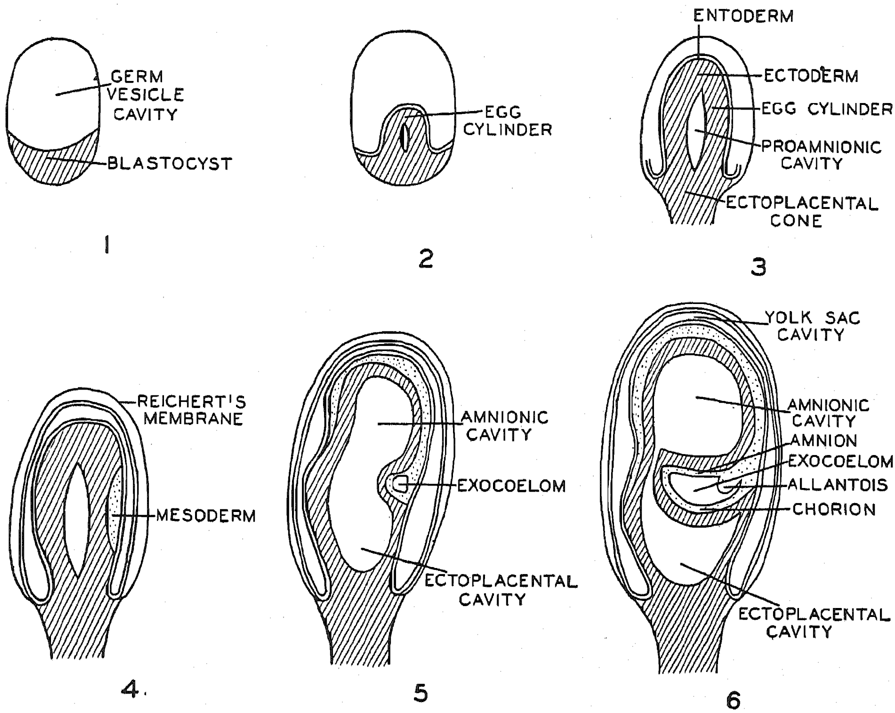
A short account of the early embryogeny of the mouse is given here for comparison with the description of the abnormalities in the homozygote t^0t^0.

The early stages of the embryogeny of the mouse up to the eighth day after fertilization have been described very thoroughly by LEWIS and WRIGHT (1935) (preimplantation stages) and by SOBOTTA (1895, 1903, 1911). Our own observations start at the time of implantation of the embryo, and agree perfectly with those of SOBOTTA.

Fertilization takes place in the Fallopian tube. During the first and later stages of cleavage the egg stays in the oviduct. The two cell stage is attained at about 24 to 36 hours, the four to eight cell stage at 44 to 50 hours, the eight-cell to morula stage at 50 to 69 hours. The egg is very small, and the morula consists of very small cleavage cells. At about the 32 cell stage (that is, approximately the beginning of the fourth day) the eggs enter the uterus and begin to disperse throughout it. In this stage (the second half of the fourth day) the cleavage cavity within the egg forms; it is multiple, eccentric and irregularly shaped. At about the beginning of the fifth day the cleavage cavity of the blastocyst becomes rounded off and regular, and the blastocyst is now spherical.

From now on a considerable increase in cell number takes place, the germ vesicle becomes ellipsoid and the largest part of the wall of the germ vesicle touches the decidua of the uterus. This stage is reached on about the first half of the fifth day (figure 1). Implantation does not take place until the end of the fifth day. Up to that time development is very slow. Only after implantation (that is, after one third of the entire gestation period has passed) does development begin to proceed with great speed. From this stage on, SOBOTTA'S observations have been confirmed by our own. Discrepancies in the determination of the age of the embryos are probably due to the fact that we timed matings within a shorter time limit than SOBOTTA did.

At the end of the fifth day the mesometral wall of the germ vesicle begins to thicken and the cells begin to grow both into the germinal cavity in the shape of a cone and into the lumen of the uterus (figure 2). The cone-shaped mass of cells becomes the egg cylinder. The cells of the egg cylinder displace the former germ cavity; this is the beginning of the phenomenon known as entypy or inversion of germ layers, which results in the ectoderm becoming the innermost and the entoderm the outermost layer of the em-



FIGURES 1–6.—Diagrams of different stages in early mouse development, from implantation stage to formation of mesoderm. For detailed description see text.

bryo. At the mesometral pole of the blastocyst the ectoplacental cone is formed (figure 3). In the egg cylinder a cavity develops (figure 3). The entire cylinder and the inner wall of the germ vesicle are now covered by one layer of yolk entoderm. At about the end of the seventh or the beginning of the eighth day the first mesoderm appears at the posterior end of the future embryo between the external and internal cell layer of the egg cylinder (figure 4). Thus the wall of the egg cylinder consists of three layers at this place. In sections of the egg cylinder the division of the egg cylinder cavity into amnionic and ectoplacental cavities begins to become apparent (figure 5 and 6). Both of these cavities are still connected with each other at this stage.

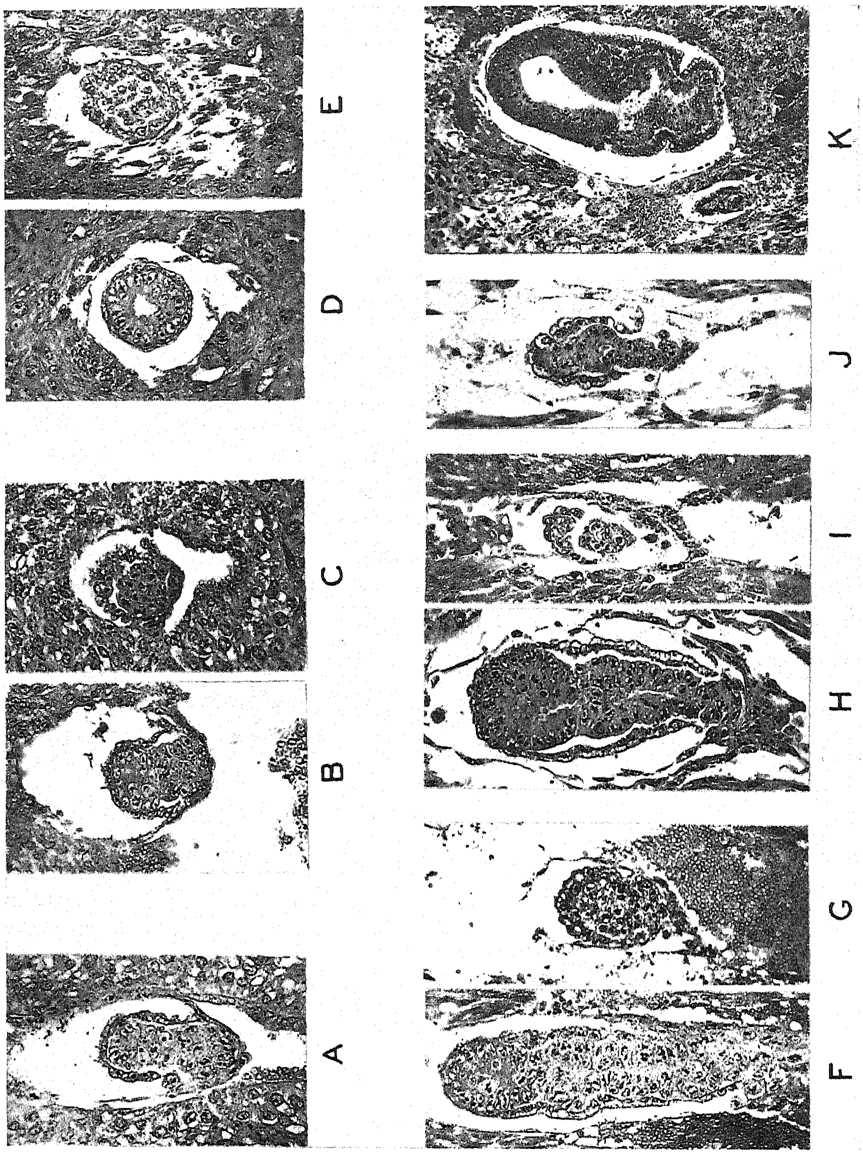
HISTOLOGICAL DESCRIPTION OF THE HOMOZYGOUS EMBRYOS (t^0t^0)

Among 32 embryos from three early litters (aged four days, 23 hours to five days, three hours) only three abnormal embryos were found. These three which were dead and in the process of being resorbed, had apparently died from accidental causes. At this early stage (the end of the fifth day) the embryo has just become implanted. Plate 1, figure A gives a typical picture of such a young embryo in the early egg cylinder stage. It has not been possible so far to detect any differences between normals and typical abnormals at this early stage. In a litter dissected five days five hours after copulation the earliest typical differences between normals and abnormals were observed. Figures B and C both show oblique sections through a normal (B) and an abnormal (C) embryo of this litter. There are two main differences to be observed between the two types. First, the normal embryo shows the typical differentiation of the ectoderm into two spherical cell masses while the abnormal embryo has an undifferentiated ectodermal center mass. Second, the entodermal layer of the normal embryo is thin and regular, while that of the abnormal embryo is thick and irregular; the individual entoderm cells of the abnormal embryo are larger and have more cytoplasm than the normal entoderm cells.

Figures D and E show the differences between normals and lethals to have become more obvious at a slightly older stage. Figure D represents a cross section through the egg cylinder of a normal embryo, five days 20 hours ± three hours after copulation. Figure E shows a cross section through its abnormal litter mate. The abnormal embryo is not dead yet, as the finding of a mitosis indicates; the difference from the normal can be found in the disorganization of the ectodermal cell mass (for example, the absence of the first trace of the proamnionic cavity) and in the disorganized appearance of the outer (entoderm) layer.

Figures F and G show two littermates—one normal, the other abnormal—in longitudinal sections. The normal embryo is in the stage of the elongated egg cylinder. Mesoderm formation has not occurred as yet. The abnormal embryo is much smaller than the normal and consists of a uniform inner cell mass which is surrounded by a heavy layer of very large entoderm cells. This picture reminds one of a very early normal stage (figure A) except for the thickness of the entoderm and the disorganization of the inner cell mass. It looks as if the embryo had stopped developing at the very early egg-cylinder stage and abnormal processes had then set in which resulted in thickening of the entoderm and disorganization of the ectodermal cell mass.

Figures H and I show longitudinal sections through embryos slightly older than those pictured in figures D and E, figure H being a section of a normal embryo in a stage just before the appearance of mesoderm, figure I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that of an abnormal litter mate. This latter picture is quite typical of the abnormal type in this stage: there is an ectodermal cell mass with living cells in the center (presence of mitoses) and an entodermal cell cap consisting of many large entoderm cells with a great amount of cytoplasm. While in the stage preceding this, entoderm and ectoderm are still in close contact, these two layers have now separated. The entoderm "cap" has been lifted off the ectodermal cell mass. This phenomenon, although found frequently in this stage, is not regularly observed.

The development of the normal embryo proceeds at a rapid pace at this time. The egg cylinder elongates, and mesoderm appears; the amnionic folds begin to form and the formation of the proamnionic cavity, ectoplacental cavity and exocoelom proceeds.

While all these developments go on in the normal embryo within approximately 24 hours, the picture of the lethal embryo does not change very much. There is always an inner cell mass surrounded by a heavy entoderm layer with the entoderm cells being very large and proliferating most strongly at the antimesometral pole of the embryo (figure J). Sometimes this entodermal "cap" is lifted off as shown in figure I, but sometimes the outer entoderm stays in close contact with the inner cell mass. More and more degenerated and granulated cells are found in the abnormal embryo, until by the end of the seventh day nothing but a mass of pycnotic granules is left.

Figure K represents a curious phenomenon encountered in sections of one of the embryonic capsules, dissected at the age of seven days, three

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Description of Plate 1

Photographs of sections of mouse embryos showing effects of lethal $t^{o}t^{o}$.

Figure A: 4918,4l. Age 5 days 3 hours ± 1 hour. Longitudinal section through embryo in the young egg cylinder stage. 250×.

Figure B: 4907,3r. Age 5 days 5 hours ± 1 hour. Oblique section through normal embryo. 250×.

Figure C: 4907,2r. Age 5 days 5 hours ± 1 hour. Oblique section through abnormal embryo, litter mate of figure B. 250×.

Figure D: 4908,5r. Age 5 days 20 hours ± 3 hours. Cross section through normal embryo. 250×.

Figure E: 4908,6l. Age 5 days 20 hours ± 3 hours. Cross section through abnormal embryo, litter mate of figure D. 250×.

Figure F: 4108,3l. Age 6 days ± 1 hour. Longitudinal section through normal embryo. 250×.

Figure G: 4108,4r. Age 6 days ± 1 hour. Longitudinal section through abnormal embryo, litter mate of figure F. 250×.

Figure H: 4071,1l. Age 5 days 21 hours ± 1 hour. Longitudinal section through normal embryo. 250×.

Figure I: 4071,2l. Age 5 days 21 hours ± 1 hour. Longitudinal section through abnormal embryo, litter mate of figure H. 250×.

Figure J.: 4138,4l. Age 6 days 3 hours ± 3 hours. Longitudinal section through abnormal embryo. 250×.

Figure K: 4021,1l. Age 7 days 3 hours ± 4 hours. Longitudinal section through two embryos, implanted next to each other in utero, one normal (right), the other abnormal (left). 120×.

hours ± four hours. Two embryos, one normal, the other lethal, had become implanted next to each other in the uterus. Thus, the picture of a section through these two embryos presents an excellent demonstration of the differences between normal and lethal embryos. The difference in size is obvious; while the normal embryo shows the amnionic cavity, the amnionic folds and the appearance of mesoderm between the inner (ectodermal) and the outer (entodermal) layer, the lethal embryo shows the typical abnormal picture: an unorganized inner cell mass in which mitoses were found, with a heavy entoderm layer around it. Some cells at the top of the cylinder begin to disintegrate.

DISCUSSION

From the histological description of the development of the t^0t^0 embryo it appears that normal and lethal embryos cannot be distinguished before the age of five days. The very young egg cylinder stage is identical for both normals and lethals. But while the normal embryo soon begins to grow rapidly and morphogenesis proceeds, growth and development of the abnormal embryo cease. Until about the age of six and a half days, the lethal embryo is characterized by an unorganized inner cell mass surrounded by a heavy entoderm layer; the whole embryo has the shape of a young egg cylinder. This unorganized cell mass begins to show signs of degeneration around the age of six and a half days; it soon becomes necrotic and finally is resorbed.

The death of the embryos in the A line at about the age of seven days is due to their genetic constitution and not to accidental causes. A comparison of the data presented here with those obtained in the 29 line (GLUECKSOHN-SCHOENHEIMER 1938b), corroborates this conclusion. In the 29 line, in which death of t^1t^1 occurs before implantation, 21 litters dissected at the age of seven and eight days, contained 152 embryos of which 146 were normal and six (four percent) were abnormal. Dissections of 10 litters in the A line at the corresponding age yielded 101 embryos of which 60 were normal and 41 (40.6 percent) were abnormal. Since the A line and the 29 line have the same genetic background (Bagg albino) and differ chiefly in the genes t^0 and t^1, the great number of abnormal and dead embryos at the age of seven and eight days in the A line must be attributed to the lethal action of t^0t^0. The eight (2.9 percent) atypical abnormals found in the A line and mentioned above may be comparable to the four percent of abnormals found in the 29 line.

In the study of the development of the tailless mouse Tt^0 it was found that notochord, mesoderm and hindgut were affected in the embryo. These structures—according to SOBOTTA's papers—can be traced back to the re-

gion of the primitive gut and it was suggested that a malformation in the region of the primitive gut might give rise to the malformations described for the tailless mouse. The fact that the homozygous lethal t^0t^0 stops its development just before the time of primitive gut and mesoderm formation suggests that a fundamental disturbance in organization occurs in this abnormal embryo. Perhaps one might be permitted to call this disturbance a complete failure of gastrulation, gastrulation in the mouse being the process of formation of primitive gut, primitive streak and mesoderm.

CHESLEY was able to show that T has an effect primarily on the notochord. From the present study it appears that t^0 may act primarily on the mesoderm.

One may attempt to correlate in the following manner the different malformations and their development in the different genotypes carrying T and t^0. In the heterozygous Brachy ($T+$) mouse the notochord in the posterior region of the embryo is defective and as a result the Brachy phenotype develops. In the tailless mouse Tt^0, the notochord and the mesoderm of the posterior region of the embryo are defective as a result of which the phenotype of the tailless mouse develops. In the homozygous Brachy mouse (TT) the entire notochord is affected; this embryo dies on the 11th day of development. The t^0t^0 homozygote—described in this paper—fails to form any mesoderm; the embryo dies on the seventh day of development, that is, soon after the beginning of mesoderm formation in the normal. It is impossible to decide whether the lack of mesoderm formation and of further differentiation is the cause of death, or whether the failure to form mesoderm and to differentiate are secondary to some abnormal condition of the embryo which eventually leads to its death. In the latter case the lack of mesoderm and the failure to differentiate would be only two phenomena of the entire syndrome. Only evidence from transplantation experiments could reveal the role that the mesoderm (or the lack of mesoderm) plays in the development of the abnormal embryo.

The effect of the two alleles T and t^0 on notochord and mesoderm might suggest that the two alleles act on two *different* structures. However, if considered from the embryological point of view, the notochord and mesoderm of the mouse have the same origin, namely in the tissue of the wall of the primitive gut. Since the t^0t^0 homozygote does not live until the time of notochord formation, it is not possible to decide whether it would be able to form notochord; the complete failure of formation of mesoderm therefore excludes the possibility of notochord formation.

KOBOZIEFF and POMRIASKINSKY-KOBOZIEFF, in a recent paper (1939) report that dissections of uteri of pregnant females from tailless by tailless matings gave two classes of lethals, and dissections from $+t^0$ by $+t^0$ mat-

ings, one class of lethals. This agrees with our findings. The authors do not mention the time of death nor any detailed examination of the abnormal embryos.

SUMMARY

The development and time of death of the t^0t^0 homozygote are described.

The t^0t^0 homozygote can first be distinguished from its normal litter-mates at the age of about $5\frac{1}{4}$ days after fertilization.

At this age the development of the homozygote stops. It remains alive for about 40 hours, without showing any signs of growth, organization or mesoderm formation.

At about seven days after fertilization necrosis of the homozygous embryo sets in and resorption takes place.

From the study of the development of Tt^0 and t^0t^0 it is concluded that t^0 affects development and formation of mesoderm.

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CYTOGENETIC STUDIES ON TRITICALE. I. A METHOD FOR DETERMINING THE EFFECTS OF INDIVIDUAL SECALE CHROMOSOMES ON TRITICUM[1]

J. G. O'MARA

U. S. Department of Agriculture, Columbia, Missouri

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THE production of fertile, true-breeding hybrids which combine the characters of two different species or genera has proven to be of theoretical and practical importance. These hybrids have usually arisen through a doubling of the chromosome complement following the initial cross, this doubling resulting in the presence in the fertile hybrid of two full complements from each parent. Such plants are known as amphidiploids because the chromosome set of each of the two parents is present twice, or in the diploid condition. It is possible, however, to combine characters of two species or genera by a method which involves the addition of one or more chromosomes from one species to the full diploid complement of the other species. When the chromosomes of one species have desirable and undesirable dominant characters distributed among the various chromosomes of the set, these characters are given to the second species without selection in the amphidiploid. If only the chromosomes with the desired dominant characters could be added to the chromosome complement of the second species, the value of the combination for theoretical and practical purposes would be greatly enhanced. Such combinations have been secured between Secale and Triticum, and the method by which they were obtained should be applicable to other genera where an amphidiploid is available.

The method, in brief, involves the production of an amphidiploid between two species or genera, A and B. The amphidiploid is then crossed to one of the parents—for illustration, species A. The individual obtained from this latter cross would have two full sets of chromosomes from species A and one full set from species B. During meiosis, the two sets from species A would undergo normal pairing and distribution, whereas the set from species B would remain as univalents and would be distributed at random. Thus the gametes from such a plant would contain a full set of chromosomes from species A, and from none to a full set from species B. From such a plant the offspring would contain two full sets of chromosomes from species A and from none to various combinations of the chromosomes of species B.

[1] These studies are a part of the program supported by funds obtained under Bankhead-Jones Project SRF-2-5, "Comparative Genetics and Cytology of Polyploidy Series in Triticum," Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and Missouri Agricultural Experiment Station, cooperating.

CHROMOSOME BEHAVIOR OF THE PARENTAL TYPES

The amphidiploid used in these experiments (produced by E. R. SEARS) consisted of spring rye, *Secale cereale*, and "Chinese" spring wheat, *Triticum vulgare*, and was derived directly from the first generation hybrid by duplication of the chromosomes in somatic tissue. These three types may be seen in figure 1, where a is the rye, c the wheat, and b the amphidiploid. The wheat-rye hybrid has been studied cytologically by KIHARA (1924), THOMPSON (1926), LONGLEY and SANDO (1930), FLORELL (1931), and KATTERMANN (1934). KIHARA reported that the hybrid, at metaphase 1, had from one to three bivalents, and his report was confirmed by THOMPSON, who found the same numbers of bivalents, and who further reported that he believed the bivalents to be the result of autosyndesis among the wheat chromosomes. LONGLEY and SANDO observed no pairing in this cross but found the same number of bivalents, from one to three, in a hybrid involving *Secale montanum*. FLORELL observed that in the wheat-rye hybrid, involving Hybrid 128 wheat and Rosen rye, from one to three bivalents were formed, approximately half the cells having two bivalents. KATTERMANN reported as many as six bivalents in some cells. The amphidiploid between wheat and rye has been made several times, and its cytology has been most thoroughly investigated by MÜNTZING (1939). All the amphidiploids reported thus far have been asynaptic to some degree, univalents occurring at metaphase 1. The amphidiploid used in these experiments had approximately 43 percent of the cells without univalents. No Triticale strain with a perfectly regular meiosis has been reported. As a result of this irregularity, variations occur in the chromosome numbers of the gametes and of the progenies.

For the purposes of the present method of procedure, the production of a plant with two full complements of wheat chromosomes and one full complement of rye chromosomes is absolutely necessary. An amphidiploid sector on a wheat-rye hybrid was backcrossed to wheat. A single plant from this cross was examined cytologically and found to have 21 normally-behaving pairs of wheat chromosomes and seven univalent rye chromosomes. This plant was allowed to self, and from it 70 seeds were obtained.

THE PROGENY OF THE PLANT DIPLOID FOR WHEAT AND HAPLOID FOR RYE CHROMOSOME COMPLEMENTS

The 70 seeds obtained from the plant which had two wheat complements and one rye complement produced 70 plants. These plants had 21 pairs of wheat chromosomes and various numbers of rye chromosomes, ranging from one to seven. The presence of rye chromosomes in these plants produced very striking effects on the morphology of the plants. All the rye chromosomes occurred as univalents, which were distributed in the random fashion usual for univalents.

One plant among the 70 is interesting in that it had 28 univalent chromosomes at the first meiotic division. Presumably, this plant resulted from the parthenogenetic development of an egg which had received the full set of wheat chromosomes plus all seven of the rye chromosomes. This plant, then, should have the same constitution as a first-generation wheat-rye hybrid, since it had a haploid set of wheat and a haploid set of rye chromosomes.

Twenty-nine of the 70 plants were selected for carrying on the rye chromosomes to the next generation. These plants were quite fertile, possessed certain rye characters, and showed 21 pairs of normally-behaving wheat chromosomes plus one or two rye univalents at metaphase I. The plants were selfed by bagging the heads before anthesis. From the 29 progenies of these plants, a single plant which carried the character under consideration, was selected from each of 18 progenies, and these 18 plants were selfed. The selfing was carried on, in this generation, to reduce the number of rye chromosomes to one. Since the rye chromosomes were present as univalents, they tended to be lost at meiosis for two reasons: (1) univalents are often lost in the cytoplasm at meiosis, where they form micronuclei; (2) each univalent is distributed to but half the gametes, and so the number decreases in successive generations. These 18 plants consisted of nine plants which had hairy-neck, two which had tapered heads, and seven which had awns. These characters were selected for study because their distribution among the 70 plants indicated that they were in different chromosomes. The progenies of the 18 plants were examined cytologically.

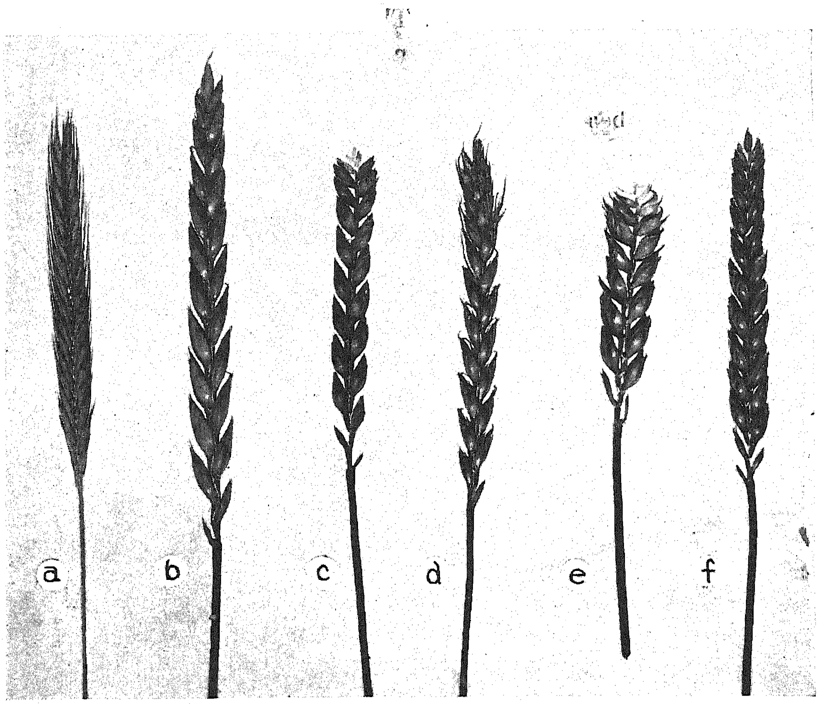
In the progenies of eight of the nine hairy-neck plants, approximately one third of the individuals had hairy-neck. In the progeny of the remaining hairy-neck plant, consisting of 19 individuals, all the plants had hairy-neck. Upon examination, all these 19 individuals proved to have at least 22 pairs of chromosomes, that is, 21 wheat bivalents plus one rye bivalent. Three of the 19 plants had 23 pairs of chromosomes. In the progenies which were segregating for hairy-neck, all but two of the affected plants had the rye chromosome present as a univalent. These two plants had 22 pairs of chromosomes. They were disomic for the rye chromosome and similar in appearance to the plants of the progeny which had all 22-chromosome plants. The rye chromosome, when disomic, did not always form a bivalent but was present in a small percentage of cells as two univalents, and when present as a bivalent, often separated when the wheat chromosomes were at anaphase.

The hairy-neck chromosome had a marked effect upon the plant, other than the characteristic pubescence just below the head. Since all the plants which had the character were nearly identical in appearance, it is very probable that but one of the rye chromosomes carries the factor or factors involved in producing the character. Those plants which were monosomic

for this chromosome were slightly shorter than normal wheat plants and had heads with broader, coarser spikelets. When the chromosome was disomic, these alterations were exaggerated, and the plants were approximately two thirds the height of normal wheat, and had coarser, broader spikelets than the plants monosomic for the chromosome. These differences may be seen by comparing c, a normal head, with e, a head disomic for the hairy-neck chromosome, in figure 1. A comparison of the amounts of pubescence on the necks of plants monosomic and disomic for the rye chromosome and on the neck of the amphidiploid is interesting, in that such a comparison shows that the amphidiploid has the least pubescence, although it has the rye chromosome present in two doses. This comparison would seem to indicate that no accurate predictions of the degree of manifestation of any character can be made from the amphidiploid. The derived type may be more like one parent than like the amphidiploid, although both have identical doses of the chromosome concerned.

The rye chromosome which carries the factors or factor producing tapered head, imparts to the individual possessing it a head without any compacting at the tip, such as is characteristic of the wheat parent. The two progenies having this character were found to be different, one retaining several extra rye chromosomes. This one was not studied. The other progeny consisted of 30 plants, five of which had the character. Four of these five were monosomic for the chromosome, and the other plant was disomic for it. This rye bivalent was less stable than the hairy-neck bivalent, occurring in many cells as two univalents. The univalents can always be identified as rye chromosomes—and not wheat—by their larger size and consistent form. The effect of this chromosome on the plant, other than that of producing the tapered head, was to increase the height of the plant so that individuals carrying the chromosome as a monosome were slightly taller than normal wheat plants, and the plant which carried the chromosome as a disome was over a third taller. The effect of this chromosome, therefore, seemed to be additive, since the disomic was taller than the monosomic plant. The difference, however, was not so marked as with the hairy-neck chromosome.

The term "tapered" was applied to this character because the heads of the affected plants did not have the compacted tip characteristic of the parental wheat, but tapered continuously from basal to apical spikelets. A comparison of head c—which is normal—and f, which carries the chromosome as a disome, will show the difference. At least one other rye chromosome, however, has a similar effect, and the term was used for this chromosome because no clearly separable effect, other than "tapered," was detectable, whereas another chromosome which produces the same effect carries, in addition, a factor for producing awns.



DESCRIPTION OF FIGURE I

Heads of plants of various chromosome constitutions. ($\times\frac{3}{5}$)

a. *Secale cereale* involved in amphidiploid illustrated at b.
b. Amphidiploid involving a, *Secale cereale*, and c, *Triticum vulgare* var. "Chinese spring."
c. *Triticum vulgare* var. "Chinese spring" involved in amphidiploid illustrated at b.
d. Triticum to which has been added the Secale chromosome producing awns. The chromosome is present as a disome.
e. Triticum to which has been added the Secale chromosome producing hairy-neck. The chromosome is present as a disome.
f. Triticum to which has been added the Secale chromosome producing tapered-head. The chromosome is present as a disome.

The seven progenies from awned plants did not fall into distinct classes; five were segregating, with approximately one third of the individuals awned, one had seven awned plants and two normal wheat plants; and one progeny consisting of 13 individuals had all awned plants. The segregating progenies had an extra univalent chromosome; and the group with all awned plants had an extra bivalent his bivalent seemed to be fairly regular in behavior, and although it sometimes separated late, most cells at anaphase 1 had 22 chromosomes at each anaphase pole.

The chromosome ring the factor or factors for awns had the most striking effect on the plant, especially when disomic. The plant is usually shorter than normal wheat, has no compacting of spikelets at the tip, has many fine, dark green leaves when young, is inclined to be spreading in habit, and possesses awns approximately one fifth the length of those of the rye parent. The awns are not uniform throughout the head, being nearly absent from some spikelets, and generally longest at the tip of the head, as may be seen in d of figure 1.

In one segregating progeny a plant was discovered which had 23 bivalents and one univalent. This plant had both awns and hairy-neck, so that two of the three chromosomes represented may be accounted for by phenotypic effects. No effect of the other chromosome or chromosome pair was observable.

In addition to the progeny of the single plant secured by backcrossing the amphidiploid to the wheat parent, the progeny of one other plant of similar parentage was grown. Of this progeny seven plants were examined cytologically. One of these had 22 pairs of chromosomes plus two univalents,—an instance which indicates that the bivalents may be added directly in the progeny of the backcrossed plant, although none of the original 70 plants had such an extra bivalent.

THE INTRODUCTION OF RYE BIVALENTS

Thus far all attempts to secure rye characters in wheat-like plants have been made by using the F_1 hybrids in backcrosses to wheat. This method has, in some respects, proved quite practicable, as the results of LEIGHTY and TAYLOR (1924), of FLORELL (1931), of TAYLOR (1939), of KATTERMANN (1934), (1935), (1937), (1938), and of LEDINGHAM and THOMPSON (1938) indicate. Any additions to the basic wheat complement obtained by this method may, however, consist of wheat chromosomes or chromosome pairs and not of rye, as LEDINGHAM and THOMPSON concluded from their studies on progenies of wheat-rye hybrids backcrossed to wheat. KATTERMANN'S extensive studies on plants secured by this method of using the F_1 hybrid indicate that the female gametes of the hybrid may have from 17 to 31 chromosomes. While variations occur in the gametes of the am-

phidiploid, none of this order are indicated by the data of MÜNTZING (1939). The hybrid, from a cytological viewpoint, at least, is comparable to a haploid; and SEARS' (1939) studies on the progeny of a haploid wheat indicate that duplications of whole chromosomes occur in the female gametes. These duplications in the hybrid should involve wheat chromosomes three times as often as rye. Further, the haploid was found by SEARS to produce very aberrant progeny—monosomes, trisomes, and segmental interchanges—all of which would usually be undesirable where the process of addition is to be carefully controlled.

The origin of the present wheat plants disomic for a rye chromosome is capable of two interpretations. Since none of the 70 original plants—secured from the plant with two wheat complements and one rye complement—had pairs of rye chromosomes, these must have occurred in a later generation. That the chromosome is not usually transmitted through the pollen may readily be concluded, since in segregating progenies disomic plants are rare; and no pairs were found among the 70 plants where a maximum probability exists for finding pairs. These disomic plants, however, may occasionally occur as the result of a monosomic pollen grain competing successfully with normal pollen. The other possibility is that in a plant with a single rye chromosome non-disjunction—or some other meiotic anomaly—may occur to produce a disomic egg which, when fertilized by a normal pollen grain, would produce a disomic plant. Either of these methods is possible, and each may be tested by appropriate backcrosses to normal gametes of eggs and pollen from monosomic plants.

Should these disomic plants prove not to be true-breeding—and so resemble those of LEDINGHAM and THOMPSON—they should nevertheless, by chance distribution, make available monosomic and disomic plants in appreciable numbers in succeeding generations.

Another method could be used to secure these results. If the plants with rye univalents were pollinated by the amphidiploid, each univalent would be supplied with its homologue. Those chromosomes without homologues would be eliminated by successive selfings.

The failure of the rye chromosomes to pair as regularly as the wheat chromosomes is difficult to explain. It may represent some inability of rye to operate normally in a cell which is almost wholly wheat. The tendency to asynapsis may also be due to the absolute homozygosity of the rye chromosome and the consequent uncovering of recessive factors which have deleterious effects on the regularity of meiosis. However, all such factors now known affect meiosis as a whole and not merely the single chromosome which bears them.

USES OF THE DERIVED TYPES

These plants with single chromosomes from one species added to the full unaltered chromosome complement of a wholly different species, have several obvious practical and theoretical uses. They could be used to determine efficiently what factors dominant to the genome upon which they were superposed were linked together in the single chromosome. By crossing two plants homozygous for different chromosomes, the interaction of these two known chromosomes could be studied. By using the plants in which single chromosomes had been isolated, various tests for disease resistance, hardiness, and various other factors important economically, could be made to determine if the trait desired were located in a single chromosome or were a property of the whole complement. Such information should indicate whether any single property desirable in one genome could be transferred to the other through rare non-homologous pairing or through induced translocations. Such useful transfer is obviously impracticable where the desirable character is a property of the whole genome or of many factors located in different chromosomes.

ACKNOWLEDGMENT

The author is indebted to DR. E. R. SEARS, who furnished the original seed of the amphidiploid and its backcross which were used in these experiments, and to Dr. L. J. STADLER and DR. BARBARA MCCLINTOCK for suggestions during the preparation of the manuscript.

SUMMARY

By backcrossing an amphidiploid to one of its constituent species and selfing for several generations, individuals were produced with a single chromosome pair from one species in the amphidiploid added to the unaltered chromosome complement of the other species. These additions represent three different rye chromosomes, phenotypically detectable, which have been added to an unaltered wheat genome. The method should be applicable to other genera where an amphidiploid is available and may have some value in practical and theoretical genetics.

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THE GENETICS OF SEX DETERMINATION IN THE FAMILY AMARANTHACEAE*

MERRITT J. MURRAY
Cornell University, Ithaca, New York[1]

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INTRODUCTION

AS HAS been emphasized by YAMPOLSKY (1922, 1925) angiospermous plants exhibit many physiological as well as morphological forms of sex expression. Many of these forms appear to be intermediate steps in the evolution of one type into that of another, as has been suggested by CORRENS (1928) and others. EMERSON (1924), JONES (1934), and LEBEDEFF (1938) have predicted that the many gene mutations which affect sex in maize are the building blocks that could lead to the development of a different type of sex expression in this plant. In fact, EMERSON (1932) and JONES (1932, 1934) have produced dioecious strains of maize by the proper combinations of two genes.

Is the evolution of dioecious forms from hermaphroditic ones, or the reverse, explainable on such a simple genetic basis? CORRENS (1907, 1928) early attempted to solve this question by direct hybridization, but the sterility of his F_1 hybrids did not allow him to continue his analysis beyond the first generation. Recent work on cultivated species and varieties indicates that sex expression is markedly influenced by a few gene mutants having very specific actions. STOREY (1938 a, b) and HOFMEYR (1938) believe that a single gene with two alleles will explain much of the sex variability within the species *Carica Papaya*. OBERLE (1938) found that variations in the sex expression of Vitis species, varieties, and hybrids is best explained on the basis of two allelic pairs of factors which are completely linked. ROSA (1928) has shown that a monoecious condition in muskmelons is dominant to an andromonoecious condition and that this difference is due to a single factor. POOLE and GRIMBALL (1939) have shown that monoecious and perfect-flowered varieties of muskmelons differ in two gene mutations and that the perfect-flowered form is genetically the double recessive type. Do these same conclusions apply to natural species which have evolved two different but stable sex forms?

The work reported in this paper represents a search for suitable material which would allow a direct genetic analysis of second generation progeny

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resulting from the hybridization of dioecious and hermaphroditic forms. Members of the family Amaranthaceae were selected at PROFESSOR R. A. EMERSON's suggestion, since he had observed in nature sterile F_1 hybrids arising from the natural hybridization of a monoecious and a dioecious species. As a large number of closely related monoecious and dioecious species are available in this group of plants, certain combinations might be found which would give fertile hybrids, unless there is some direct relation between sterility and the evolution of sex differences. A detailed account of this work is given here. Preliminary reports in abstract form have already been published (MURRAY 1938, 1939, 1940 a, b).

MATERIALS AND METHODS

Species which have been used successfully in crosses are given in table 1. Numerous pollinations were also made between this material and species in other genera such as Celosia, Gomphrena and Achyranthes but no hybrids were obtained.

TABLE 1

Origin, sex, and chromosome number of the species used in this study.

SPECIES	ORIGIN	SEX	N CHROMOSOME NUMBER
Amaranthus hybridus L. Angol race	Near Angol, Chile	Monoecious type 1	16
Amaranthus hybridus Line 58	Ithaca, N. Y.	Monoecious type 1	16
Amaranthus hybridus Line 56	Ithaca, N. Y.	Monoecious type 1	16
Amaranthus caudatus L. var. leucospermus (Wats.) Thell. Mexican race	Mexico	Monoecious type 1	16
Amaranthus caudatus L. var. leucospermus (Wats.) Thell. Manchurian race	Manchuria	Monoecious type 1	16
Amaranthus retroflexus L.	Ithaca, N. Y.	Monoecious type 1	17
Amaranthus Powellii Wats.	Kansas		
	Ithaca, N. Y.	Monoecious type 1	?
Amaranthus spinosus L.	Central Yucatan	Monoecious type 2	17
	Belle Glade, Fla.		
Acnida tamariscina (Nutt.) Wood	Lincoln, Neb.	Dioecious	16
Acnida cuspidata Bert.	Canal Point, Fla.	Dioecious	16
Acnida tuberculata Moq.	Westerville, Ohio	Dioecious	16

The material was identified by DR. P. C. STANDLEY of the Field Museum of Natural History who has monographed this family. It is well known by taxonomists that this is, at least in part, a difficult group and that the correct application of names in some cases requires study of type specimens present in European herbaria. To know the exact material used in this study, reference may be made to specimens deposited in the Cornell University herbarium or the Field Museum of Natural History, Chicago.

The three races of *Amaranthus hybridus* have been treated as though they were species since they are morphologically distinct in habit, leaves and inflorescence characters. They breed true to type and at least part of their hybrid combinations are almost completely sterile.

While taxonomic manuals disagree in their descriptions of the sex of the several members of the genus Amaranthus, the races and species which I have used are strictly monoecious. The arrangement of the staminate and pistillate flowers within the inflorescence is a very definite one. This may be seen from a consideration of the growth pattern shown by the species used in this work The main axis of the inflorescence is usually branched.

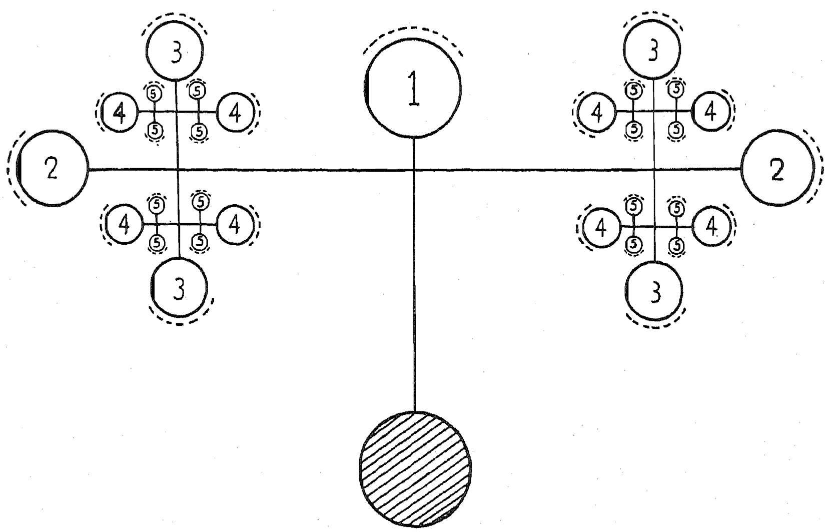

FIGURE 1.—Diagrammatic illustration of the arrangement and development of individual flowers within a flower cluster. A broken line indicates the position of the bract which subtends each flower. The shaded circle is the main axis of the inflorescence.

The length and number of these branches and their angle with the main axis determine the shape of the inflorescence. Individual flower clusters develop along these axes in an alternate fashion, while within each flower cluster, individual flowers are produced as illustrated diagrammatically in figure 1. The first flower is terminal on the branch and at its base, two branches develop the second and third flowers. Each of these flowers in turn is terminal and at its base develop the next two flowers. This process continues until all the available space is occupied. Development is usually very symmetrical up to the third or fourth series of flowers. At this time the setting of the first seed usually slows down growth and upsets the symmetry. Unpollinated clusters may develop as many as 250 flowers. My diagram has purposely exaggerated distances between the flowers and has also

placed the branches exactly opposite each other as they appear to be in gross dissections. The apparently opposite branching in the flower cluster is probably produced by shortening the axes of an alternate branching system.

This growth pattern is common to all the species regardless of sex. In the dioecious species, the flower clusters not only have all their flowers of the same sex but all the flower clusters on one plant are of the same sex. The monoecious species exhibit two types of arrangement of the staminate and pistillate flowers. These types are important because of their different breeding behavior.

In the first type, the first flower of each flower cluster is staminate and all the succeeding ones are pistillate. There is only one staminate flower in each flower cluster of the inflorescence and this abscisses soon after shedding pollen. The monoecious species of Amaranthus except *Amaranthus spinosus* belong to this group.

In the second type, all the flowers of each cluster are of the same sex but the clusters of pistillate flowers develop only in the axils of the branches and at the base of the terminal inflorescence while the clusters of staminate flowers are borne terminally on the main axis and lateral branches. *Amaranthus spinosus* is of this type.

All species used in this study are wind-pollinated. The monoecious members are chiefly self-pollinated, although the stigmas of the pistillate flowers are receptive several days prior to the opening of any staminate flowers. The small, closely grouped flowers of the monoecious species made emasculation extremely difficult. The most satisfactory method of making crosses onto the monoecious species is to pollinate heavily as soon as the stigmas are receptive and to remove the staminate flowers by hand. Even so, 5–25 percent self-pollination usually occurs. The hybrids are easily distinguished from the monoecious parent and in several crosses dominant genes have been used to distinguish the hybrids in the seedling stage.

Six kinds of crosses have been made and will be discussed in the order given below.

1. Interspecific crosses of dioecious species of Acnida.
2. Interspecific crosses of monoecious species of Amaranthus.
3. Intergeneric crosses of dioecious species of Acnida and monoecious "first type" species of Amaranthus.
4. Intergeneric crosses of dioecious species of Acnida and monoecious "second type" species of Amaranthus.
5. Backcross of F_1 hybrids obtained from "3" to both parental types.
6. Backcross of F_1 colchicine-induced tetraploid hybrids obtained from "3" to both parental types.

RESULTS

Interspecific crosses of dioecious species of Acnida

Three different dioecious species breed true to type and give normal 1:1 sex ratios (table 2). All six possible F_1 hybrids have been made and each of these gives a 1:1 sex ratio, with the possible exception of the first cross which shows a deviation slightly greater than should be expected by chance.

TABLE 2

Hybridization results of interspecific crosses of dioecious species of Acnida.

CROSS	FEMALE	MALE	D./P.E. 1:1	D./P.E. 1:2
A. Control—Dioecious species inbred:				
Acnida tamariscina (Nutt.) Wood	2259	2291	.75	
Acnida cuspidata Bert.	72	64	1.02	
Acnida tuberculata Moq.	43	39	.66	
B. First generation progeny of interspecific crosses:				
A. tamariscina×*A. cuspidata*	752	664	3.47	
Reciprocal	622	568	2.32	
A. tamariscina×*A. tuberculata*	83	100	1.97	
Reciprocal	58	62	.54	
A. cuspidata×*A. tuberculata*	173	179	.47	
Reciprocal	80	102	2.42	
C. Second generation progeny of interspecific crosses:				
F_2 (*A. tamariscina*×*A. cuspidata*)	196	167	2.3	
B.C. (*A. tamariscina*×*A. cuspidata*)× *A. tamariscina*	67	137		.22
F_2 (*A. cuspidata*×*A. tamariscina*)	73	79	.72	
B.C. (*A. cuspidata*×*A. tamariscina*)× *A. tamariscina*	71	84	1.66	
B.C. (*A. cuspidata*×*A. tamariscina*)× *A. tamariscina*	49	116		1.46

The second generation progeny consist of normal male and female plants. Certain crosses give 1:1 sex ratios while other crosses give ratios of one female to two males. The latter type of cross is due apparently to the presence of a recessive sex-linked lethal carried in the X chromosome. If this is true, the genotypes of the F_1 parents are X^lX^+ and X^lY^+. The male is known to be the heterogametic sex from genetic evidence presented later in this paper.

No intersexes, hermaphroditic plants, or sex abnormalities were found in the second generation progeny obtained from reciprocal hybridization of these two different dioecious species. Why should this be true? If individual chromosomes of these two species carry different quantitative amounts of

male and female determining factors (due either to the quantitative nature of a few genes or to the number of these genes), why does not the normal distribution of the chromosomes in the F_1 hybrids result in the production of plants with an abnormal balance of sex factors? The segregation of two monofactorial differences furnishes genetic evidence that at least one or two chromosomes segregate in a normal manner and there is no evidence suggesting that other members behave differently. Two hypotheses to explain the above data are cited.

First, if sex is determined by a balance between many sex determiners which act in either a male or a female direction, we must almost certainly conclude that these two species have, chromosome for chromosome, an identical balance of these factors. This assumption seems unreasonable from an evolutionary viewpoint. Two species which differ primarily in quantitative characters probably have had genic changes in every chromosome (MURRAY 1940a). Major changes in the chromosome arrangement of the genic material have apparently occurred. Why should genes affecting size and shape of morphological structures mutate while genes influencing sex do not? If a primitive dioecious Acnida plant came originally from a hermaphroditic ancestor, we could postulate a mass mutation phenomenon to account for this fact. The further assumption follows that once this balance of many factors is created, stability of all or most of the sex factors results, even though mutation of other types continues. The dioecious and monoecious species of this group of plants are certainly closely related; otherwise one could hardly account for the 33 different intergeneric hybrids that have been obtained. The explanation just attempted becomes very complicated and may be disregarded as a much simpler hypothesis can explain the same data.

A few gene mutations of a very specific nature could occur and cause the evolution of a dioecious form from a hermaphroditic one. EMERSON (1932) and JONES (1932, 1934) have shown that this can be accomplished experimentally in maize, using only two mutants which have occurred spontaneously. Their examples have one mutant gene homozygous in both sexes; the second gene is homozygous in one sex and heterozygous in the other. These two factors are located on different chromosomes. The first gene may be termed an autosomal factor; the second gene creates the XX and XY mechanism. If all monoecious maize were suddenly exterminated and these dioecious races were saved, one would not be able to detect the presence of the autosomal gene. One might discover that one sex was homogametic and the other heterogametic.

The same reasoning may be applied to the sex mechanism in Acnida species. The autosomes either carry no genes influencing sex or carry them in a homozygous condition. If autosomal sex genes are present, they are prob-

ably few in number, since two dioecious species do not differ in this regard although they exhibit other major changes of chromosome structure and genic content. This discussion is concerned only with those factors which differentiate between hermaphroditic species and closely related dioecious species. There is abundant evidence in plants and animals that many loci on most or all of the chromosomes are concerned with the physiology and morphology of reproductive structures, but only those genes which are different in the two sex forms need enter into this discussion.

SCHAFFNER (1923) observed *Acnida tamariscina* and found that the sexual states of this species were extremely stable. I, too, have found that this species exhibits very few sex abnormalities, even under the extreme conditions of low light intensity and low temperature. Occasionally a staminate plant will produce a few flowers having a partially developed ovary. These more or less perfect flowers do not produce seeds as they wither and absciss soon after the pollen is shed. No abnormalities have been observed on pistillate plants.

Although the reversion of 101 females and 243 males to the vegetative state was observed, only one example of complete sex reversal has been found. This plant originally had about 25 male flowers. After growing vegetatively, the plant was entirely female and normal in every respect. Crosses made with this plant as a female gave 400 ♀♀ : 385 ♂♂ which shows that the plant had a female genotype even though a few male flowers were produced at one time.

Interspecific crosses of monoecious species of Amaranthus

All monoecious species and races breed true and have been inbred one to three generations before being used in crosses. The two types of arrangement of the staminate and pistillate flowers found in these monoecious species (table 1) are phenotypically very stable. Abnormalities are rarely encountered.

Thirteen crosses have been made between members possessing the first type of sex arrangement (table 3) and the hybrids, thus obtained, have the same sex arrangement. This result would be expected if all these species are homozygous for the factors controlling this particular positional placement of the staminate and pistillate flowers. These species hybridize readily but the F_1 hybrids are so highly sterile that F_2 plants are seldom obtained. The few that were secured did not show segregation. On the other hand, the high sterility makes it easy to get amphidiploid progeny following colchicine treatment of the growing point. When the treatment is effective, an abundance of seed is produced; otherwise, only a few seeds would be procured from an entire inflorescence. The five amphidiploids have the same sex arrangement as their parents.

TABLE 3

Hybridization results of interspecific crosses of monoecious species of Amaranthus.

CROSS OR SELF	MONOECIOUS	REMARKS
A. Control—Monoecious Amaranthus species selfed	Breed true to type	
B. Interspecific crosses of monoecious species:	(Number of plants)	
First type×first type:		
A. hybridus Angol race×*A. caudatus* Manchurian race	548	1st type
Reciprocal	119	1st type
A. hybridus Angol race×*A. caudatus* Mexican race	28	1st type
A. hybridus Angol race×*A. hybridus* Line 58	166	1st type
A. hybridus Angol race×*A. hybridus* Line 56	169	1st type
Reciprocal	71	1st type
A. hybridus Angol race×*A. Powellii*	124	1st type
A. hybridus Angol race×*A. retroflexus*	434	1st type
Reciprocal	363	1st type
A. hybridus Line 56×*A. Powellii*	1	1st type
Reciprocal	1	1st type
A. hybridus Line 58×*A. Powellii*	5	1st type
Reciprocal	15	1st type
A. hybridus Line 58×*A. retroflexus*	1	1st type
A. retroflexus×*A. Powellii*	9	1st type
Reciprocal	1	1st type
A. caudatus Mexican race×*A. retroflexus*	115	1st type
Reciprocal	5	1st type
A. caudatus Mexican race×*A. Powellii*	61	1st type
A. caudatus Manchurian race×*A. hybridus* Line 58	172	1st type
First type×second type:		
A. caudatus Manchurian race×*A. spinosus*	2	1st type
A. retroflexus×*A. spinosus*	2	1st type
A. hybridus Angol race×*A. spinosus*	20	Intermediate
C. F_2 progeny of interspecific crosses of monoecious species:		
F_2 (*A. retroflexus*×*A. caudatus* Mexican race)	3	1st type
F_2 (*A. retroflexus*×*A. spinosus*)	10	2nd type
D. Amphidiploid progeny of interspecific crosses of monoecious species:		
F_2 (*A. hybridus* Angol race×*A. retroflexus*)	71	1st type
F_2 (*A. caudatus* Mexican race×*A. retroflexus*)	106	1st type
F_2 (*A. caudatus* Manchurian race×*A. hybridus* Line 58)	242	1st type
F_2 (*A. caudatus* Manchurian race×*A. hybridus* Angol race)	49	1st type
F_2 (*A. hydridus* Angol race×*A. hybridus* Line 58)	16	1st type

Members of the two types of sex arrangement hybridize with great difficulty. The plants that have been obtained are certainly hybrids as they show two dominant leaf color genes carried by the male parent. The first type is completely epistatic to the second type when the species *A. caudatus* and *A. retroflexus* are crossed to *A. spinosus*. An intermediate condition

TABLE 4

F_1 hybridization results of intergeneric crosses of dioecious species of Acnida and monoecious "first type" species of Amaranthus.

CROSS	♀♀	♂♂	D./P.E. 1:1
Acnida cuspidata×*Amaranthus retroflexus*	8	0	
Reciprocal	287	282	0.37
A. cuspidata×*A. hybridus* Angol race	717	0	
Reciprocal	453	411	2.31
A. cuspidata×*A. hybridus* Line 58	53	0	
Reciprocal	133	113	1.89
A. cuspidata×*A. caudatus* Mexican race	124	0	
Reciprocal	53	48	0.89
A. cuspidata×*A. caudatus* Manchurian race	73	0	
Reciprocal	144	121	2.19
A. cuspidata×*A. Powellii*	40	0	
Reciprocal	—*	—	
Acnida tuberculata×*Amaranthus retroflexus*	—	—	
Reciprocal	82	92	1.12
A. tuberculata×*A. hybridus* Angol race	346	0	
Reciprocal	276	314	2.32
A. tuberculata×*A. hybridus* Line 58	—	—	
Reciprocal	87	98	1.31
A. tuberculata×*A. caudatus* Mexican race	—	—	
Reciprocal	42	52	1.53
Acnida tamariscina×*Amaranthus retroflexus*	547	0	
Reciprocal	339	374	2.00
A. tamariscina×*A. hybridus* Angol race	1070	0	
Reciprocal	1536	2007	11.72
A. tamariscina×*A. hybridus* Line 58	251	0	
Reciprocal	191	173	1.40
A. tamariscina×*A. hybridus* Line 56	4	0	
Reciprocal	4	3	1.12
A. tamariscina×*A. caudatus* Mexican race	728	0	
Reciprocal	517	410	5.26
A. tamariscina×*A. caudatus* Manchurian race	805	0	
Reciprocal	315	429	6.20
A. tamariscina×*A. Powellii*	17	0	
Reciprocal	—	—	

* Hybrids not studied; but readily obtainable.

results when *A. hybridus* is crossed to *A. spinosus*. These hybrids also have normal flowers but the staminate and pistillate flowers bear no precise positional arrangement with reference to each other. This condition is apparently related to certain factors present in the *A. hybridus* genom which interact only with certain factors in the genom of *A. spinosus* since F_1 hybrids between *caudatus*, *retroflexus*, and *hybridus* show no peculiarities.

Intergeneric crosses of dioecious species of Acnida and monoecious "first type" species of Amaranthus

The results obtained from reciprocal crosses between three different dioecious species and several monoecious first type species are summarized in table 4. All 29 of these intergeneric crosses are consistent in showing that dioeciousness is epistatic over monoeciousness. Fourteen of these crosses were made using the dioecious species as the female parent and these crosses give all female hybrids. The reciprocal crosses, where the monoecious species is the female parent, give ratios of approximately 1 ♀ : 1 ♂. These results are in agreement with CORRENS' (1907) work on Bryonia. His interpretation that the male is the heterogametic sex is likewise true for the three species of Acnida.

All crosses which involve the dioecious species *Acnida cuspidata* and *Acnida tuberculata* give progenies consisting exclusively of normal female hybrids and the reciprocal crosses give 1:1 sex ratios well within the expected deviations. This is not true of certain crosses with *Acnida tamariscina.* The summarized ratios, from certain of those crosses which segregate for males and females, exhibit deviations from a 1:1 ratio much greater than would be expected by chance. Many of the hybrids do not produce flowers and thus there is omitted from table 4 a considerable number of plants whose sex could not be determined. An analysis of individual crosses with consideration given to the non-flowering plants is necessary for an understanding of this problem.

Neuter plants. Plants which do not bear flowers will be designated in this paper as neuter plants. They are indistinguishable from normals in so far as the vegetative parts of a plant are concerned. Neuter plants develop an inflorescence devoid of the actual flowers. The bracts which subtend normal flowers are present but not even minute rudiments are externally visible to indicate that the flower primordium began development. Presumably because the flowers are not present to make nutritional demands on the plant, the individual axes within each flower cluster continue to grow rapidly and a large, crowded, bract-filled inflorescence results. The only fundamental difference is the absence of flowers, as the growth pattern is unchanged.

Neuter plants occur only in the hybrids arising from crosses of the dioecious species *Acnida tamariscina* to any of the monoecious first type species. It must be emphasized that the monoecious species which are used in these crosses are normal in appearance and had been inbred one to three generations. Likewise, *Acnida tamariscina* progeny were grown in considerable numbers without finding any sexual abnormalities and all crosses were made on plants which were inbred one or two generations. It was thought (MURRAY 1938) that the neuter plants were due to an environmental effect on an unstable hybrid complement. An analysis of a larger number of

crosses now shows that the type of segregation depends on the particular Acnida male or female plant used in the cross. Individual plants of either sex give hybrid progenies with no neuter plants, fifty percent neuter plants, or all neuter plants (table 5).

TABLE 5

Types of segregation for neuter plants in reciprocal crosses of Acnida tamariscina to the monoecious "first type" species.

Acnida tamariscina	NUMBER OF PLANTS IDENTIFIED	F_1 PROGENY ♀♀	♂♂	NEUTER	D/PE 1:1	χ^2 1:1:2
As female parent:						
Free from neuter	10	1923	0	6*		
Heterozygous for neuter	12	1255	0	1230	.74	
Homozygous for neuter	4	4*	0	292		
As male parent:						
Free from neuter	2	406	510	0	5.09	
Heterozygous for neuter	9	1316	1499	2035	5.09	138.9
Homozygous for neuter	1	1*	0	462		

* Presumably off-pollinations or mechanical mixture of seeds.

The original race of *A. tamariscina* obtained from nature must have been heterozygous for a single dominant factor which induces neuterness when outcrossed to any of the monoecious species. Differences, even in chromosome number, between the monoecious species have no effect on the segregation of neuter in the resulting hybrids. Proof of this comes from the fact that at least three monoecious species with differences in chromosome numbers have been crossed to the same Acnida male or female plant in each of the six classes of segregations found in table 5.

There is a considerable deficiency in the number of neuter progeny in the cross of monoecious by heterozygous male, as one should expect a ratio of 1 ♀ : 1 ♂ : 2 neuter assuming that the factor is independent of sex. This deficiency is probably not to be ascribed to a lower viability of the neuter plants since in the reciprocal cross the neuter progeny appear with the expected frequency. In these same crosses the frequency of the males is out of proportion to that of the females, although not more so than in the cross where neuters are not present. The neuter-inducing factor inhibits the development of flowers of both sexes although not always in equal proportions. These discrepancies are even greater when one considers the results from any one male plant. The variability encountered includes such extreme ratios as 62 ♀♀ : 331 ♂♂ : 310 neuter and 424 ♀♀ : 307 ♂♂ : 548 neuter. A ratio of 271 ♀♀ : 278 ♂♂ : 408 neuter is much more nearly that expected if there is no linkage with sex. Pollen grains carrying the neuter

factor appear not to function as frequently as those carrying the recessive allele. No satisfactory explanation of these deviations is possible at the present time.

If a female plant of *Acnida tamariscina* known to be free of the neuter factor is crossed to an *A. tamariscina* male known to be heterozygous for the factor, the F_1 male plants segregate for bad pollen. Twelve plants had good pollen and eleven had about 50 percent pollen abortion. Likewise, if a female plant known to be homozygous for the neuter factor is crossed to a male plant known to be heterozygous, the F_1 males segregate. Twenty-seven F_1 plants had good pollen and 12 plants had about 50 percent pollen abortion. These records indicate that the neuter-inducing factor when heterozygous is associated with 50 percent pollen abortion and probably with 50 percent ovule sterility. A cytological study has not yet been made to ascertain whether a chromosomal change is actually present.

Flower color. The species *Acnida tamariscina* exhibits variations in plant color and an attempt was made to study these in the hope of obtaining a sex-linked character. Crosses of entirely green-stemmed plants to brilliant red-stemmed ones gave apparently heterozygous intermediate red-stemmed plants. When one of these heterozygous females is crossed to a monoecious species, linkage with the neuter-inducing factor is found. The results are given in tabular form below:

Red ♀	Green ♀	Red neuter	Green neuter
1	322	344	4
182	5	4	153

Since these are essentially backcross progenies, the two linkage phases may be added together in the calculation of the percent of recombination (1.4 percent).

The results of a second generation analysis of plant color in the species *Acnida tamariscina* are difficult to interpret. They suggest that the interaction of two autosomal genes are responsible for the type of plant color. It has been shown that one of these genes is closely linked to the neuter-inducing factor and that the latter is probably related to a chromosomal change. While an unknown translocation or inversion by itself ought not to disturb the ratios, partial linkage with a lethal would. This seems to be true. The neuter factor may be considered an autosomal factor since the closely linked color factor does not show sex linkage.

Neuter hybrids occasionally have small chimeras in the inflorescence and these areas bear either normal staminate or pistillate flowers. In those families where the neuter hybrids are all modified females these chimeras have pistillate flowers. In other families, where the neuter hybrids may be either modified males or females, some of the plants have pistillate

FIGURE 2. a (upper left).—Normal *Acnida tamariscina* (dioecious). Male at left, female at right. b (upper right).—*Amaranthus hybridus* Angol race, a monoecious first type species. c (lower left).—F_1 female (left) and male (right) hybrids resulting from the cross of *Amaranthus hybridus* Angol race by *Acnida tamariscina* male. d (lower right).—F_1 neuter hybrid resulting from the same cross.

FIGURE 3. a (upper left).—F_1 neuter hybrid resulting from the cross of *Amaranthus hybridus* Angol race by *Acnida tamariscina* male. Arrow points to chimera of normal male flowers. b (upper right).—Viable F_1 male hybrid resulting from cross of *Acnida tamariscina* by *Amaranthus spinosus*. Picture includes only one branch of a plant five feet tall. c (center).—Barely viable F_1 male hybrid resulting from the same cross. Note the size of this complete plant ($1\frac{1}{4}$ inches tall) of approximately the same age as the plant figured in b. d (lower left).—*Amaranthus spinosus*, a monoecious second type species. Arrow indicates division line between female (below) and male flower clusters (above). Note lighter V-shaped area on leaf due to presence of a dominant gene. e (lower right).—F_1 monoecious hybrid resulting from cross of *Amaranthus hybridus* Angol race by *Amaranthus spinosus*. No definite positional arrangement of the staminate and pistillate flowers.

chimeras, others staminate ones. The sex of the F_1 hybrids is quite stable in that staminate and pistillate flowers are rarely found on the same plant. The only observed exceptions were a few neuter plants which had both staminate and pistillate chimeras. Figure 3a illustrates a striking example of a large chimera where normal male development occurs on one side of a neuter plant. A cytological study of the male flowers of this chimera showed that development was halted before division I. It was therefore impossible to learn whether one or more chromosomes had been lost somatically.

One family not included in table 4 or 5 was a cross of *Acnida tamariscina* by *Amaranthus hybridus* Angol race. This cross gave 3 female to 11 monoecious plants. The flower arrangement of the monoecious hybrids was identical to that of the monoecious parent and somatic chromosome counts on one monoecious hybrid did not reveal any deviation from the expected number of 32 chromosomes. Apparently the particular Acnida female used in the cross was heterozygous for a mutation affecting sex, but unfortunately no other crosses were made on the same plant and this type of cross has not reappeared. Since part of these plants were grown in the greenhouse and part in the experimental plot, it is unlikely that this change was due solely to environmental causes. All the hybrid plants were pollinated but produced no fertile seed.

BRESSMAN (1934) has noted a high percentage of non-flowering (neuter) hop plants in certain cultures. Whether there is any real similarity between the neuter plants found in Acnida and hops cannot be determined from the published facts.

Intergeneric crosses of dioecious species of Acnida and monoecious "second type" species of Amaranthus

Amaranthus spinosus does not hybridize as readily with the dioecious species as do the monoecious first type species. The first progenies of these hybrids consisted entirely of small plants which usually died in the seedling stage. The fourth cross in table 6 is an example. Twenty-two plants out of 350 survived until they flowered and these grew very slowly for five months and never attained a height of more than two inches. This non-viability is associated with certain crosses and not with others. The first three families (table 6) indicate that progenies may be obtained consisting of all non-viable seedlings, 50 percent non-viable seedlings, or all viable seedlings. The dominant factor responsible for non-viability in the hybrids is probably carried in the dioecious species, *Acnida tamariscina*, since the *Amaranthus spinosus* plants came from a line inbred for two generations. In fact, this non-viability factor may be identical with the factor which induces the development of neuter hybrids in crosses with the monoecious first type species.

Disregarding non-viable progeny, males predominate in the cross dioecious by monoecious where all females would be expected if the monoecious parent had been one of the first type species. In addition to the males a limited number of monoecious plants are produced. In the reciprocal cross, males also predominate. Apparently male-determining factors present in the monoecious genom are epistatic over female-determining factors present in the dioecious genom.

TABLE 6

F_1 hybridization results of intergeneric crosses of dioecious Acnida species and Amaranthus spinosus, the monoecious "second type" species.

CROSS	♀♀	♂♂	MONOECIOUS	REMARKS
Acnida tamariscina×*Amaranthus spinosus*	0	347	3	No loss of seedlings
Acnida tamariscina×*Amaranthus spinosus*	6	207	41	50% loss of seedlings
Acnida tamariscina×*Amaranthus spinosus*	0	3	0	±100% loss of seedlings
Amaranthus spinosus×*Acnida tamariscina*	1	17	4	All are type usually dying as seedlings
Amaranthus spinosus×*Acnida tamariscina*	0	186	4	No loss
Amaranthus spinosus×*Acnida cuspidata*	0	3	3	No loss
Amaranthus spinosus×*Acnida tuberculata*	0	52	7	No loss

It is difficult to know how much significance should be attached to the occurrence of the monoecious plants enumerated in table 6. Pistillate flowers are developed sporadically in the lowermost axils of these plants and vary in number from a few flowers to a maximum not exceeding 5 percent of the total. While a hybrid with 10,000 staminate flowers and only 25 pistillate flowers may be called monoecious, it is not strictly comparable to its monoecious parent which has an equal number of staminate and pistillate flowers. A study of phenotypic expression gives one the impression that factors which localize the development of the staminate flowers at the ends of the inflorescence branches in *A. spinosus* are lax in their effects in the hybrids and allow the production of staminate flowers throughout the plant with the possible exception of the lowermost axils which ordinarily do not produce flowers. Stem cuttings taken from the base of a hybrid plant, sometimes at least, produce an abundance of female flowers. The few female plants are most difficult to explain on a genotypic basis. Furthermore, the three monoecious hybrids found in the cross of *Amaranthus spinosus* by *Acnida cuspidata* approach the normal monoecious parent much more closely in phenotypic expression. One of these plants was a tetraploid.

Backcross of F_1 hybrids obtained from "3" to both parental types

The primary aim of this work was to find a hybrid combination of stable dioecious and monoecious species which would be fertile enough to enable

one to study backcross progenies. The dioecious species of Acnida have a haploid chromosome number of 16, the monoecious species of Amaranthus 16 or 17. This indicates that *Amaranthus hybridus* and *caudatus* are more closely related to Acnida than are *retroflexus* or *spinosus*. Yet all cross readily with Acnida to give F_1 hybrids. All F_1 hybrid combinations exhibit about 99 percent pollen abortion and the ovule sterility must be equally as great, although this is more difficult to estimate. A cytological examination of the microsporocytes of one intergeneric hybrid showed that chromosome distribution in the first division is very unequal and is even more unequal in the second division. This fact by itself could account for the high sterility. It is interesting that fertility in the hybrids is restored concurrently with the doubling of the chromosome complement. This indicates that failure to synapse properly is probably the major cause of this sterility.

TABLE 7

Backcross of F_1 hybrid females (derived from intergeneric crosses) to parental types.

CROSS	♀♀	♂♂	NEUTER	MONOECIOUS NOR-MAL	MONOECIOUS SPO-RADIC	D/PE 1:1
Plot E and F. Backcross to dioecious parent						
F_1 (*Acnida tamariscina*×*Amaranthus hybridus* Angol race)×*A. tamariscina*	488	520	15	0	6	1.50
F_1 (*Acnida tamariscina*×*Amaranthus retroflexus*) ×*A. tamariscina*	37	52	15	0	0	2.52
Plot A. Backcross to monoecious parent						
F_1 (*Amaranthus hybridus* Angol race×*Acnida tamariscina*)×*A. hybridus* Angol race	255	14	3	7	9	
F_1 (*Acnida tamariscina*×*Amaranthus hybridus* Angol race)×*A. hybridus* Angol race	1558	328	114	42	40	
Control. Normal *Acnida tamariscina*×*A. hybridus* Angol race	125	5	14	0	0	
Plot C. Backcross to monoecious parent						
F_1 (*Acnida tamariscina*×*Amaranthus retroflexus*) ×*A. retroflexus*	54	0	0	6	0	
F_1 (*Amaranthus retroflexus*×*Acnida tamariscina*) ×*A. retroflexus*	263	0	1	3	1	
F_1 (*Amaranthus retroflexus*×*Acnida cuspidata*) ×*A. retroflexus*	6	0	0	0	0	

Use of the pollen from the male hybrids is not feasible, because the flowers usually do not open sufficiently to protrude the anthers, and the anthers usually fail to dehisce. As preliminary backcrossing attempts using the female hybrids yielded so few seeds per pollination, the use of isolation plots was necessary. Ten to 25 female hybrids were planted in each plot

together with the pollen producer which was either the dioecious or the monoecious parent. As noted previously, new flowers are continually produced in unpollinated inflorescences or where the seed set is poor. Consequently, a large hybrid plant at the end of the season may have had as many as a hundred thousand flowers and so would yield some seed despite the high sterility. Results from these backcross progenies are summarized in table 7.

Backcrosses to the dioecious parent (plots E and F) in the two cases studied give ratios of 1 ♀ : 1 ♂ with no more than the usual deviation. This result was expected because these are backcrosses to the epistatic sex condition. The neuter plants are due to the dominant factor carried in the dioecious parent, *Acnida tamariscina*. No significance can be attached to the proportion of neuter hybrids since several male plants were used as pollen producers. Part of these may have been free from this factor, part heterozygous for it, and part homozygous for it.

The results from backcrossing the F_1 female hybrids to the monoecious parent are shown in table 7, plot C. The results are essentially the same regardless of which way the F_1 cross was made, but the high proportion of females and low proportion of monoecious plants was not anticipated. If the segregation of chromosomes in the hybrid was completely random, gametes containing all possible combinations of the two chromosome complements should be secured. While many of these might be lethal, one should expect at least three kinds of functional gametes; namely, those having an entire genom originally obtained from the dioecious parent, those having an entire genom originally obtained from the monoecious parent, and those having both genoms. The first type of hybrid gamete after fertilization by a monoecious gamete should reconstitute the F_1 hybrid female, the second should reconstitute the monoecious parent and the third should produce a triploid plant with two monoecious genoms and one dioecious female genom. One can be reasonably certain that the third type of gamete is functional, otherwise doubling the chromosome complement of these hybrids ought not to restore fertility. The second type of gamete must be formed very infrequently because the monoecious plants, which occur, are sterile and phenotypically unlike the parental monoecious species. One cannot be sure about the first type of gamete since the phenotypic appearance of the resulting F_1 hybrids might be indistinguishable from the triploids. These second generation plants are remarkably uniform in all characters and while similar in appearance to the F_1 hybrids, their pubescence, leaf shape and habit approach more closely the characters of the normal monoecious parent, *Amaranthus retroflexus*. Somatic chromosome counts were made for 16 plants selected at random from the cross F_1 (*Acnida tamariscina* × *Amaranthus retroflexus*) × *Amaranthus retroflexus*.

All plants had the triploid number of 50 chromosomes which would be attained by the combination of one dioecious Acnida genom of 16 chromosomes with two monoecious Amaranthus genoms of 17 chromosomes each. Likewise, nine plants from the cross F_1 (*Acnida tamariscina*×*Amaranthus hybridus*)×*Acnida tamariscina* have the expected triploid number of chromosomes, $(16+16+16)=48$. This is proof that the F_1 hybrids produce only one principal type of viable gamete and that this gamete contains both genoms.

Progenies derived from plot A crosses differ from plot C crosses in having a considerable number of male and neuter plants. There must have been some dioecious pollen present in this plot since the control had five males. Chromosome counts of several females selected at random show that these plants have the expected triploid number of 48 chromosomes obtained from backcrossing to the monoecious parent. However, one neuter and one male definitely had 48 chromosomes which could be attained from backcrossing to the dioecious parent. Thus, the male and neuter plants found in these families probably should be disregarded. An equal number of the females should have been due to pollination with dioecious pollen. The corrected figures for plot A are 241 ♀ ♀ :16 monoecious or a ratio of 15:1 and 1230 ♀ ♀ :82 monoecious or a ratio of 15:1. In these two crosses the F_1 hybrid female plants came from reciprocal crosses. Plot C has 323 ♀ ♀ :10 monoecious or a ratio of 31:1.

It is difficult to evaluate the significance of these ratios and the presence of the monoecious plants. They have a near triploid constitution but this may not be a balanced complement. In so far as the vegetative parts of the plant are concerned, monoecious plants are indistinguishable from triploid females. The production of stamens is limited to the first flower of each flower cluster exactly as in the monoecious first type species. Ovary development may or may not be inhibited in these staminate flowers and this varies on the same plant. Some plants have all the first flowers of each flower cluster staminate or perfect (called normal monoecious); others have only a few of the first flowers staminate or perfect (called sporadic monoecious). With so much variability of expression, this enumeration may have little significance.

Backcross of F_1 colchicine-induced tetraploid hybrids obtained from "3" to both parental types

A 0.25 percent aqueous solution of colchicine applied to the growing points induced tetraploid chimeras on several female hybrids and these areas with restored fertility were backcrossed to the parental species. The results are summarized in table 8.

TABLE 8

Backcross of F_1 hybrid colchicine-induced tetraploid females (derived from intergeneric crosses) to parental types.

CROSS	♀♀	♂♂	D./P.E. 1:1	D./P.E. 1:2
Backcross to dioecious parent:				
F_1 (*Acnida tamariscina*×*Amaranthus caudatus* Mexican race)× *A. tamariscina*	265	311	2.8	
F_1 (*Acnida tamariscina*×*Amaranthus hybridus* Angol race)× *A. tamariscina*	26	28	0.4	
F_1 (*Amaranthus hybridus* Angol race×*Acnida tamariscina*)× *A. tamariscina*	50	49	0.3	
F_1 (*Acnida tamariscina*×*Amaranthus caudatus* Manchurian race ×*A. tamariscina*	40	86	6.0	0.6
F_1 (*Amaranthus caudatus* Manchurian race×*Acnida tamariscina*)× *A. tamariscina*	108	114	0.6	
Backcross to monoecious parent:				
F_1 (*Acnida tamariscina*×*Amaranthus caudatus* Mexican race)× *A. caudatus* Mexican race	238	0		
F_1 (*Acnida tamariscina*×*Amaranthus caudatus* Manchurian race)×*A. caudatus* Manchurian race	69	0		
F_1 (*Amaranthus caudatus* Manchurian race×*Acnida tamariscina*)× *A. caudatus* Manchurian race	6	0		

Backcrosses to the dioecious parent gave ratios of 1♀:1♂ and these plants have the expected triploid number of 48 chromosomes. Progenies derived from backcrosses to the monoecious parent consist exclusively of female plants and nineteen plants selected at random have the expected triploid number of 48 chromosomes. These backcross progenies are similar to the untreated backcrosses except that monoecious plants are not found.

DISCUSSION

It has been shown that a cross of an Acnida female of a dioecious species to a monoecious "first type" species gives only females and that the reciprocal cross gives an equality of males and females. Since the male is the heterogametic sex, the sex mechanism of these three dioecious species is, therefore, of the XY type. These crosses also give a direct comparison of the female genom X+15A to the male genom of Y+15A as the monoecious genom is identical in both cases. If the assumption is made that the autosomes exhibit random distribution in the formation of gametes and are either homozygous for all sex factors or lack of such factors, one may conclude that the sex chromosome pair (XY) contains the differential genic mechanism which initiates the development of maleness or femaleness in an individual sporophyte under normal conditions. If the auto-

somes contain sex factors and are not homozygous for them, segregation should occur in the inbred lines of the dioecious species as well as in the crosses to the related dioecious and monoecious species. However, there is no evidence of segregation either within the species or in the interspecific hybrids. If random distribution of the autosomes does not occur, abnormal ratios for two autosomal genes should be secured, but are not. This interpretation cannot be considered absolute proof that the Y chromosome carries the male-determining factors. However, if the Drosophila type of sex inheritance were true of Acnida, one should expect the XXXY ($3X+4A$) tetraploid Acnida males to be intersexes.

Since intersexes, hermaphroditic plants or sexual abnormalities are not found in the second generation progeny of an interspecific cross of two different dioecious species, the genic sex mechanism is apparently identical in both species. The interpretation is made that such exact similarity would be unlikely if sex were due to the interaction of a large number of genes.

TABLE 9

Genotypic balance in relation to sex.

DIPLOID	TRIPLOID	TETRAPLOID
Pure Species:		
d d = ♀		*d d d d* = ♀
D d = ♂		*D d d d* = ♂
		D D d d = ♂
m 1 *m* 1 = mon.₁		*m* 1 *m* 1 *m* 1 *m* 1 = mon.₁
m 2 *m* 2 = mon.₂		
Hybrids:		
d *m* 1 = ♀	*d m* 1 *m* 1 = ♀	*d d m* 1 *m* 1 = ♀
D m 1 = ♂	*d d m* 1 = ♀	*D D m* 1 *m* 1 = ♂
d m 2 = ♂	*D d m* 1 = ♂	
D m 2 = ♂	*d m* 1 *m* 2 = ♂ or mon.*	*d d m* 2 *m* 2 = mon.₂**
m 1 *m* 2 = mon.₁*		

Where *d* = ♀ genom of dioecious species; *m* 1 = genom of monoecious species first type
D = ♂ genom of dioecious species; *m* 2 = genom of monoecious species second type

* Sometimes intermediate.
** Only one plant observed.

The dioecious and monoecious "first type" species used in this work do not enable one to study directly the genetic basis of the evolution of sex. However, it is possible to compare several combinations of genoms and their relation to sex (table 9).

It is shown, in the present work, that males are formed if one male genom of Acnida is present with one monoecious first type genom of Amaranthus, and that females are formed when one female genom of Acnida is present with one or two monoecious genoms of Amaranthus and only in the absence of a male genom. MURRAY (unpublished) has shown, from a study of tetraploids of *Acnida tamariscina*, that malesness is induced when one male genom is present with one or even three female genoms.

The combination of a single female genom of Acnida with a genom of the monoecious second type species of Amaranthus usually results in maleness although occasionally the males produce a few pistillate flowers near the base of the plant. In addition a few females have been observed. It has been assumed that factors inducing maleness in the second type monoecious plant are generally epistatic to the factors inducing femaleness. When a female genom, a monoecious first type genom, and a monoecious second type genom are combined in a triploid, the result is either a male plant or a monoecious one without any precise positional arrangement of the pistillate and staminate flowers. This peculiar type of monoecism has been observed in these triploid progeny only when the monoecious first type genom is that of *Amaranthus hybridus* Angol race. The same result is obtained in a diploid hybrid when a genom of the Angol race is combined with one of the second type of monoecism. Other species of the first type of monoecious arrangement are completely epistatic over the second type; and the addition of a single female genom of *Acnida tamariscina* to these diploids results in the formation of triploid males. Whether these phenotypic effects are due to many or only a few genes cannot be stated.

The intergeneric F_1 hybrids between dioecious and monoecious first type species produce only one type of functional gamete and this contains the diploid number of chromosomes including both parental genoms. These gametes probably arise from the reconstitution of the chromosomes into one nucleus after an abortive first division. MANGELSDORF and REEVES (1939) have observed the same phenomenon in the intergeneric hybrids of Zea×Tripsacum where the only functional gametes are those possessing both genoms.

It has been shown that the presence of barely viable hybrids in the crosses of *Acnida tamariscina* to *Amaranthus spinosus* are found only in certain crosses and not in others and that their presence is probably conditioned by a single factor carried in the dioecious species. This phenomenon may be of general occurrence in wide crosses. MANGELSDORF and REEVES (1939, pp. 89, 103) have reported vigorous and dwarf F_1 hybrids in the intergeneric crosses of Zea×Tripsacum. They also cite examples in Fragaria hybrids and in sorghum-sugarcane hybrids. Since these species

are probably highly heterozygous, the same type of explanation may very well apply.

The presence of neuter hybrids in certain crosses between *Acnida tamariscina* and all monoecious first type Amaranthus species constitutes a new kind of isolation mechanism. These species will hybridize readily if growing in the same area, but hybridization could not be carried beyond the first generation since in certain lines all the F_1 hybrids are neuter plants. This mechanism is supplemented by the low degree of fertility of the hybrids which flower. It has been shown that either *Acnida tamariscina* males or females may carry the dominant neuter-inducing factor in either a homozygous or heterozygous condition, and appear phenotypically normal. Neuter hybrids are obtained only on outcrossing to one of the monoecious first type species. The factor is shown to be linked to a color gene with 1.4 percent crossing over, and when heterozygous induces 50 percent pollen abortion and presumably 50 percent ovule sterility. This suggests that the factor is probably related to, or due to, a translocation or an inversion rather than a point mutation. Phenotypically neuterness results in the suppression of flowers of either sex, although not always in the same proportion. Any direct relation to the mechanism of sex determination or evolution of sex is not apparent.

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SUMMARY

In this study of the genetics of sex determination, three dioecious species of the genus Acnida and five monoecious species (eight races) of the genus Amaranthus, family Amaranthaceae, have been used. The monoecious species exhibit two types of distribution of the male and female flowers, although the growth pattern of the inflorescence of all species, regardless of sex, is identical.

Included in this paper are the results of an investigation of 29 interspecific and 33 intergeneric F_1 hybrids; the production of tetraploid races in one dioecious and two monoecious species; the creation of five amphidiploid races; and an analysis of about 6000 second generation progeny obtained from backcrossing F_1 intergeneric hybrids to the parental species. Altogether, over 50,000 plants were used.

Genetical data indicate that the male Acnida plant is heterogametic. The hypothesis is advanced that the XY chromosome pair in the dioecious

species carries the differential sex factors and that the autosomes, if they carry sex factors, are homozygous for them not only within the species, but also in different species.

Several diploid, triploid, and tetraploid combinations of dioecious and monoecious genoms have been studied. These data show that the complex of sex factors in the Y chromosome are epistatic to those in the X chromosome and to those in a monoecious genom. Likewise, the factor, or factors, in the X chromosome are epistatic to those in the monoecious "first type" genom but are hypostatic to those in the monoecious "second type" genom.

Neuter plants, wherein the flower primordia fail to develop, occur among certain F_1 intergeneric hybrids. This condition is shown to be due to a single dominant autosomal factor present in the species *Acnida tamariscina* and *only expressed* when outcrossed to any of the monoecious "first type" species. The neuter factor is linked to a color factor (1.4 percent C.O.) and when heterozygous is associated with 50 percent pollen abortion. This suggests that the factor may be carried on a translocated or inverted region. The development of flowers of both sexes is inhibited by the neuter phenotype, so that there is probably no direct relationship to the mechanism of sex determination.

The hybridization of certain *Acnida tamariscina* plants with *Amaranthus spinosus* results in the production of barely viable hybrids. Progenies may contain all non-viable, 50 percent non-viable, or all viable hybrids. A single dominant factor, perhaps identical to the neuter-inducing factor, apparently conditions the production of barely viable hybrids. The neuter-inducing factor and also the non-viability factor (if different) are tentatively cited as new physiological types of isolation mechanisms.

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OENOTHERA CANTABRIGIANA

BRADLEY MOORE DAVIS
University of Michigan, Ann Arbor, Michigan

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OENOTHERA CANTABRIGIANA (figures 1 and 2) is a structural hybrid closely related to *Oenothera biennis* but with the interesting genetical peculiarity that the pollen and egg lethals are reversed in position from those of *biennis*. A rubens-like complex is carried by the egg and an albicans-like complex is carried by the pollen. It is as though there had been a crossover of lethals from the condition in *biennis*, but of course this cannot be assumed.

The form came to me through seed from plants which I saw growing in the University Botanic Garden, Cambridge, England in 1925. The Director of the garden, H. GILBERT CARTER, could tell me nothing of the history of the plants which may have been grown at Cambridge for a number of years. The form interested me because it differed from *biennis* in having stems red papillate and becoming light red below, midveins light red, broken streaks of red on sepals, and ovaries with red papillae (few). Because of this general development of anthocyan pigmentation, the plant has been carried in my cultures under the nickname *biennis red*.

My first culture of the plant was grown in 1928 from a collection of seeds sent to me from Cambridge. Since pollination takes place in the bud, cross fertilization is unlikely, and it was not surprising that this first culture (26.18) of 163 plants from 169 seedlings was uniform; the seed germination was 66.3 percent. From this point the race has been carried forward in a selfed line with a culture of 132 plants in 1927 and small cultures of 25–30 plants thereafter through a total of 11 generations from selfed seed. There have been no mutants in my cultures, *cantabrigiana* apparently being very stable, as is true of *biennis*.

Seed germination has been tested four times during the history of the line, the records ranging from 84.3 to 92.9 percent, a high ratio of seed fertility similar to that of *biennis*. Pollen is less than 50 percent good, *biennis* presenting a similar percentage. An examination of chromosome arrangement at diakinesis in the pollen mother cell (plant 35.18-2) showed circles or chains of ten and of four (figure 3) in contrast to the arrangement of circles or chains of eight and of six, characteristic of *biennis*.

I became aware of the position of the gamete lethals, the reverse of that in *biennis*, when in 1936–37 I grew reciprocal crosses between *cantabrigiana* and the homozygous species *franciscana*, *Hookeri* and *blandina*. The re-

Genetical Studies on Oenothera XIX. Papers from the Department of Botany, University of Michigan, No. 711.

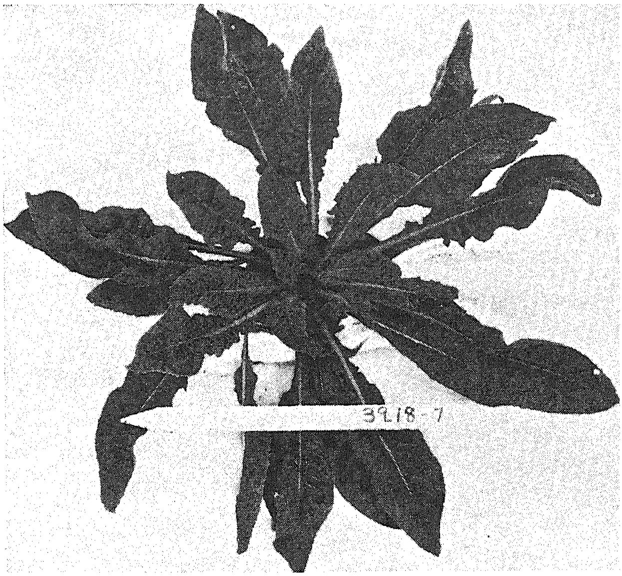

FIGURE 1.—Rosette of *Oenothera cantabrigiana.*

ciprocals resemble the Laeta and Velutina types described by DE VRIES. When *cantabrigiana* supplies the egg, the F_1 hybrid is Laeta since a rubens-like complex enters from the egg. When the pollen is from *cantabrigiana* the F_1 is Velutina because the pollen carries an albicans-like complex. The same three homozygous species crossed to *biennis* give Laeta when *biennis* as the pollen parent supplies the rubens complex and Velutina when *biennis* gives the egg with the albicans complex.

The contrasts between the Laeta and Velutina types were clear and consistent and the chief peculiarities in which there was complete agreement are given below:

	LAETA	VELUTINA
	(Egg, rubens red, from *cantabrigiana*)	(Pollen, albicans red, from *cantabrigiana*)
Rosette leaves	Broader (6–7 cm), much crinkled.	Narrower (4–5 cm), somewhat crinkled.
	Midveins red.	Midveins white.
Mature plant	Stems medium red papillate. Midveins light red. Leaves at base of plant broad (5–6 cm) strongly crinkled.	Stems strongly red papillate. Midveins white. Leaves at base of plant narrower (4–5 cm) almost plane.
Sepal tips	Slender, acute pointed, tendency to spread.	Thicker, blunt pointed, appressed.

With respect to morphological characters the contrast between bud tips is the most striking. The rubens-like complex in the egg of *cantabrigiana* brings into the Laeta type of hybrids the slender, pointed sepal tips with spreading tendencies characteristic of *biennis*. In the absence of the rubens-like complex, when *cantabrigiana* through the pollen introduces the albicans-like complex, the Velutina types of hybrids present sepal tips thicker, blunt, and appressed as found in *franciscana*, *Hookeri*, and *blandina*.

Reciprocal crosses of *cantabrigiana* to *franciscana* and *Hookeri* differ from corresponding crosses of *biennis* in one important respect: there are no classes of etiolated seedlings and rosettes which die early or produce weak plants. The cross *franciscana* by *biennis* gives a very large class of such etiolated seedlings and rosettes in sharp contrast to the few green and very vigorous plants that live. In the cross *Hookeri* by *biennis*, the seedlings are green, but the rosettes develop marked etiolation which gradually disappears in most of the plants under favorable cultivation. Corresponding crosses when *cantabrigiana* is involved are fully green and vigorous.

Description of *Oenothera cantabrigiana*

Mature rosette (Figure 1) 4–4.5 dm broad, dense, dark green. Leaves broadly elliptical, crinkled, 20–25 cm long, 5.5–6 cm broad. Midveins light red.

FIGURE 2.—Mature plant of *Oenothera cantabrigiana*.

Mature plant (Figure 2)	Central shoot 5–6 dm. Long side shoots from rosette, 5–6 dm, give a sprawling habit of growth. Stems red papillate, becoming light red below, pilose and puberulent the long hairs from the red papillae.
Foliage	Leaves at base of central shoot 17–18 cm long, 4 cm broad, somewhat crinkled. Leaves above broadly lanceolate. Midveins reddish.
Inflorescence	Bracts $\frac{1}{2}$ to $\frac{1}{3}$ length of buds.
Mature buds	6–6.5 cm long. Cone 2.3 cm. Sepals with broken streaks of red, pilose and puberulent, no red papillae. Sepal tips 3–4 mm long, slender, acute pointed, spreading. Hypanthium green.
Petals	2–2.3 cm long, golden yellow.
Stigma lobes	4 mm long, about 3 mm below tips of anthers.
Ovaries	Green with a few red papillae.
Capsules	20–23 mm long, green.
Pollen	35–40 percent good.
Seeds	Germination about 90 percent.

Chromosome configuration at diakinesis, circles or chains of ten, and of four chromosomes.

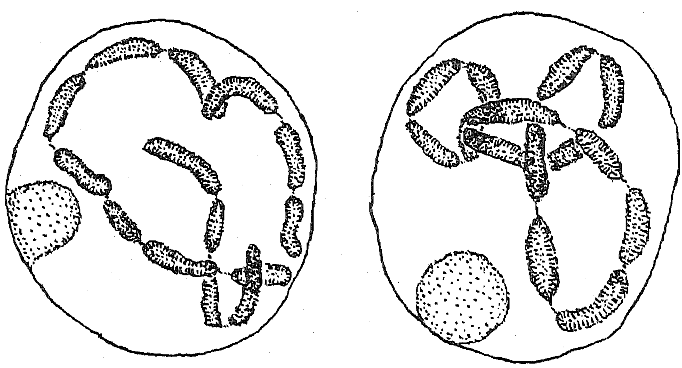

FIGURE 3.—Chromosome configurations of *Oenothera cantabrigiana*, circles or chains of 10 and of 4 chromosomes.

The discovery of the lethal situation in *cantabrigiana* has opened the possibility of obtaining two much desired plants for Oenothera genetics (1) a form close to rubens·rubens and (2) a form close to albicans·albicans. Particularly desired is rubens·rubens for the rubens complex of *biennis* has some peculiarities of genetical behavior that are not understood. At present, I am writing *cantabrigiana* as rubens red·albicans red. The pollination of *cantabrigiana* (egg, rubens red) by *biennis* (pollen, rubens) gives in the hybrid rubens red·rubens. Conversely, *biennis* (egg albicans)

pollinated by *cantabrigiana* (pollen albicans red) gives the hybrid albicans · albicans red.

These crosses have been made and the synthesized forms rubens red · rubens and albicans · albicans red have been carried into an F_3 generation. Also, certain interesting back crosses of the F_1 generations have been made. Cytological situations are being studied.

SUMMARY

Oenothera cantabrigiana is a structural hybrid closely related to *O. biennis* but with pollen and egg lethals reversed in position. A rubens-like complex is carried by the egg and an albicans-like complex by the sperm. Consequently, *cantabrigiana* crossed to the homozygous *franciscana*, *Hookeri* and *blandina* gives the Laeta type of hybrid when it furnishes the egg and the Velutina type when it supplies the pollen.

THE DISTRIBUTION OF INVERSIONS IN TRADESCANTIA

CARL R. SWANSON
Harvard University, Cambridge, Massachusetts

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THE comparative study of the cytology of species-hybrids has been a particularly fruitful one. Such analyses, dealing mostly with sterility determinations, chiasma frequencies, and chromosome configurations, have served as critical evidence for the establishment of probable interspecific, and even intergeneric, relationships, and simultaneously, have led to a better understanding of the methods by which isolation and subsequent speciation have taken place in the past, and are taking place at present. The justification for this method of approach to the whole problem of inter-relationships rests upon the assumption that the conjugation of parental chromosomes at meiosis is a relatively valid criterion of chromosome homology.

Since the advent of the salivary gland technique in Diptera, and more particularly, since the work of DOBZHANSKY (1937) and others on intra- and inter-specific hybrids in Drosophila, it has become increasingly evident that a considerable number of structural rearrangements are present which probably would never be detected in ordinary meiotic preparations. The changes are too small for legitimate pairing and crossing over to take place, and hence their presence is not revealed by the usual cytological techniques. Similarly, the regular and constant meiotic behavior in organisms with partial or complete chiasma localization may obscure profound structural changes which have occurred in genoms of the various species, and a failure to detect these differences in the hybrids is therefore no proof that such conditions do not exist. The inadequacy of cytological methods for the resolution of small structural changes when applied to plant material emphasizes the need of caution in the interpretation of relationships based on pairing in hybrids, especially those of the first generation where lower chiasma frequencies than those found in the parents are apt to be the rule. This we have found to be well substantiated by the present study.

One of the most frequently encountered meiotic aberrations in both pure and hybrid populations is that in which a portion of a chromosome is present in an inverted state. In plants this structural heterozygosity can be detected by chromatin bridges at anaphase I and II, and by the characteristic loop pairing at pachytene. In their extensive work on Tradescantia, ANDERSON and SAX (1936) conclude on the basis of chromosome behavior in F_1 hybrids that speciation in this genus has been primarily a function of gene change alone, and they make no mention of inversions

found either in the various species or in the species-hybrids which they studied. Later, SAX (1937) and DARLINGTON (1937) showed that inversions are present in all of the species observed. The frequency of this kind of structural change, as reported by them, was low with the one exception of a triploid *T. bracteata* Small investigated by SAX (1937). When the writer, in a meiotic survey of a group of diploid Tradescantia hybrids, found bridges in as high as 30 percent of the pollen mother cells of some plants, the present study was begun in an attempt to ascertain, if possible, the frequency of inversions, their general behavior and mode of transmission from one generation to another, and their possible rôle in the process of speciation within the genus.

The investigation presented in this paper, of which a preliminary report has been published (SWANSON 1940), has been a quantitative analysis of the meiotic behavior in Tradescantia insofar as it concerns inversions, chiasma frequencies, and chiasma localization. Studies have been made in the past on these three phases of meiotic activity, but they generally have been treated as separate entities. It is evident that while these three phases are superficially independent of each other, and can be treated as separate units of behaviour, there exists an interrelationship which is fundamentally important. From the results presented, it will be evident that the data obtained from the parental plants do not provide accurate information as to the kind and degree of structural change which exists between the chromosome complements of the contributing species. This is the result of a low chiasma frequency in the parental plants, and a restriction of crossing over to regions possessing little structural change. A shift in the location of crossing over toward the centromere as well as an increased range in the amount of crossing over by the segregation and subsequent recombination of genetic factors governing meiotic behavior has enabled us to reveal by a study of meiosis in the offspring, the presence of a greater degree of chromatin rearrangement than had been previously realized in this particular genus.

MATERIAL AND METHODS

The plants used in this study are two natural hybrids, called "Oak Hill 5" and "Oak Hill 13," and a number of offspring resulting from reciprocal crosses made between them. The parental plants were originally collected near Austin, Texas, and represent natural hybrids between *Tradescantia canaliculata* Rafin. × *Tradescantia humilis* Rose. They are intermediate in morphological characteristics with respect to their suspected parents. Collection and identification of these plants was made by DR. EDGAR ANDERSON of the Missouri Botanical Garden. More complete information on these species-hybrids may be obtained from ANDERSON and WOODSON'S (1935)

monograph of the genus. The Oak Hill plants are probably not strictly F_1 hybrids, since considerable back-crossing to one parent or the other may have occurred in the field and therefore their offspring do not represent a strictly F_2 generation, but it is to be emphasized that the offspring show considerable variability in growth habit, pollen sterility, and meiotic behaviour. Segregation has clearly taken place. Since the exact lineage of the Oak Hill plants can only be surmised it will be advisable to refer to them as "parental" plants, and to their offspring, collectively, as a "hybrid population."

The hybrid population composed a group of over 400 plants, only 28 of which were used in this study. Sterility determinations were made on the basis of full versus empty pollen grains, although germination tests in some cases indicate that many grains of normal appearance are non-functional. Chiasma frequencies were determined only from metaphase configurations, and all chiasma were classified as either terminal or interstitial, although the interstitial chiasmata are variable as to their location. Only strictly terminal chiasmata were classified as such. In scoring the number of pollen mother cells showing inversion bridges in both the parental and hybrid plants, it should be stated that only anaphase I configurations were counted, although bridges were also noticed frequently at anaphase II. The reason for disregarding anaphase II bridges in computing the percentages is that it has been assumed, not without evidence, that the relative bridge frequencies in the different plants would remain unchanged, since in a plant with a high bridge frequency at anaphase I, the frequency at anaphase II would be proportionately high, while a low anaphase II count would be found in a plant with a low anaphase I frequency. Chiasma and bridge frequencies for each plant were usually obtained from a single slide since environmental conditions were found to influence both of these to a considerable extent.

Meiosis in the parental plants

The general meiotic behaviour in Oak Hill 5 and 13 was essentially the same, and, with the exception of inversions, differed little from the behaviour of the Oak Hill plants which had been previously reported by ANDERSON and SAX (1936, table V, pp. 451). Metaphase configurations showed the typical Tradescantia type of pairing, that is, the bivalents were in the form of either rings or rods, with most of the chiasmata in terminal positions, but showing occasional interstitial loci paired (Plate 1, figure A). Four chiasmata per bivalent was the highest number recorded from any one bivalent configuration, giving a chiasma frequency per bivalent of 1.80 and 1.83 for Oak Hill 5 and 13 respectively. ANDERSON and SAX give frequencies of 1.6, 1.7, and 1.9 for the three Oak Hill plants

which they investigated. Inversion bridges were observed in four percent of the pollen mother cells examined in each parent (Plate 1, figure F). No more than one bridge per cell was seen in any of the preparations. The usual acentric fragment was visible in some cells, while it was invisible in others. Univalents were recorded in 20 percent of the pollen mother cells of Oak Hill 5, while Oak Hill 13 had a slightly higher percentage (Plate 1, figures C, F). Univalent formation was attributed generally to asynapsis, although some configurations suggested a premature separation of certain bivalents. Usually only a single pair of univalents was to be observed in any one cell, indicating perhaps that the same pair of chromosomes was involved each time. The certainty of this, however, could not be ascertained. The most extreme variation between the two plants was in the number of interstitial chiasmata per cell, Oak Hill 13 having about double the number that found in Oak Hill 5 (table 1). The range in variation from one cell to another was likewise greater in Oak Hill 13, several cells out of the 50 examined having as many as four interstitial chiasmata, while the other had only one cell with as many as three interstitial chiasmata. A slight variation existed in the number of terminal chiasmata per cell. The pollen sterility was approximately the same in each plant.

Meiosis in the hybrid population

Of particular interest, and that to which most attention was directed was the unexpectedly wide variation in meiotic behaviour among the plants in the hybrid population. The data are presented in table 1.

The variation in the number of terminal chiasmata per cell was small. When chiasmata counts were made on successive days, as was done in several instances for the purpose of verifying previous figures, the values were nearly identical, and showed no significant deviation. The average number of terminal chiasmata for each plant is apparently more or less constant, and is not subject to environmental variation as is the number of interstitial chiasmata. One, two, or three rod bivalents were found in almost every cell, so that cells with the maximum of 12 terminal chiasmata formed only a small percentage of the total number. The variation in the number of chiasmata at interstitial loci, however, was considerable, plant 195 having as low an average as .4 interstitial chiasmata per cell, and plant 105 having as high an average as 4.18, a variation of the order of 10:1. Figures B and C (Plate 1) illustrate some of the metaphase configurations from the hybrid population. The plants with the lowest interstitial chiasma frequency, as plants 101, 195, and 299, were quite similar in meiotic behaviour to that observed in the Oak Hill plants (Plate 1, figure A). Figures B and C (Plate 1) with eight and five interstitial chiasmata respectively are characteristic of those plants with the highest frequencies. This high

degree of interstitial exchange gives plant 105, for example, a total chiasma frequency of 14.7 per cell, or 2.45 per bivalent. There were never more than two chiasmata per chromosome arm, one terminal and one interstitial, or two interstitial.

TABLE 1

Data on bridge and chiasma frequencies. (Based on counts of at least 50 cells each.)

PLANT	PER CENT BRIDGES	INTER-STITIAL XTA/CELL	TERMINAL XTA/CELL	XTA/BIVALENT	PER CENT UNIVALENTS	PER CENT STERILITY
O.H. 5	4	.615	10.225	1.8	20	21
O.H. 13	4	1.24	9.75	1.83	26	17
101	2	.42	10.44	1.81	10	—
195	4	.4	9.52	1.50	0	very
299	4	.44	11.10	1.92	2	11
50	4.4	1.20	10.55	1.96	20	10
19	6	1.14	10.71	1.98	6	19
296	7	.91	10.32	1.87	—	24
307	7	1.06	10.06	1.85	—	13
325	8	.5	9.9	1.73	26	—
404	8	.977	10.27	1.87	—	30
257	8	1.1	9.96	1.84	—	—
251	8.53	1.96	9.8	1.96	30	13
164	10	1.03	10.18	1.85	—	15
348	10	1.20	10.56	1.96	—	10
374	11.7	1.69	10.36	2.00	11	20
302	12.2	1.38	10.97	2.06	0	15
301	13.2	1.22	10.22	1.90	—	42
255	14	1.68	11.04	2.12	2	24
42	14	2.44	10.08	2.09	12	22
223	15	2.08	9.52	1.93	—	29
265	15	2.26	9.70	1.99	—	—
220	17	2.64	9.64	2.05	—	26
359	18	2.62	10.58	2.20	—	—
382	18.24	2.65	9.97	2.08	—	—
308 (8/14/38)	20.9	2.75	10.97	2.29	—	15
308 (11/3/39)	15.6	2.56	10.91	2.24	0	—
397	21.21	2.96	10.11	2.18	—	—
105 (10/1/38)	23.2	3.42	10.26	2.28	—	—
105 (6/10/39)	32	4.18	10.52	2.45	10	17

The total chiasma frequencies of most of the hybrid population examined were higher than those of the parents, only two of them, plants 195 and 325, being lower. Four of the hybrids, plants 101, 195, 299, and 325, were lower than either parent in the number of interstitial chiasmata per cell. Previously reported chiasma frequencies of *T. humilis* and *T. canaliculata* (ANDERSON and SAX 1936), the supposed parents of the Oak Hill

plants, give *T. humilis* a frequency of 1.7 and 1.8 chiasmata per bivalent, and *T. canaliculata* 2.6 per bivalent. It would seem, therefore, that in the hybrid population frequencies lower than one parent were obtained, but that none reached that of the other parent. Realizing that the amount of germ plasm contributed by each species in the origin of the Oak Hill plants is questionable, since some backcrossing may have occurred, the comparison of chiasma frequencies is of little significance. There is no doubt, however, that the variation expressed by the hybrid population through its chiasma frequencies is the result of a segregation of genetic factors governing pairing relationships at meiotic prophase. The various recombinations are reflected in an increased range of interstitial chiasmata per cell in the hybrid population as compared to those found in the Oak Hill plants. The significance of this increased pairing will be brought out later.

Structural hybridity has been previously reported in this genus (DARLINGTON 1929, 1937, SAX 1937b). Considerable chromosome rearrangement was noted in the pollen mother cells of the hybrid population. Besides the occurrence of univalents (Plate 1, figure C), structural hybridity was evidenced through the frequent appearance of dicentric chromatids and acentric fragments at first anaphase. In none of the preparations studied were rings or chains of more than two chromosomes found, an indication that reciprocal interchanges involving the terminal ends of the chromosome arms played no rôle in the upset of meiotic regularity. Inversion bridges were found in all of the plants examined, the frequencies varying from two percent of the pollen mother cells in plant 101 to as high as 32 percent in plant 105. Most of the bridge percentages (table 1) were higher than those reported by either SAX (1937b) or DARLINGTON (1937) for diploid species and hybrids.

Generally only one bridge per cell was found (Plate 1, figures D, E, F). Occasional cells, particularly those from plants with high bridge frequencies, were found possessing two bridges (Plate 1, figure G). Several cells showed three bridges (Plate 1, figure H). These cells bearing more than one bridge were of a relatively low frequency. Only two out of about 2000 cells examined showed two bridges in the same chromosome arm (Plate 1, figure I). This could result from (1) a double crossover within a single inversion involving all four chromatids, (2) crossovers occurring in two adjacent inversions (figure 4), or (3) two crossovers in overlapping inversions (figure 5). The first condition necessitates a relatively long inversion, while the other two conditions necessitate the presence of two bridges of unequal length as well as two unequal fragments. The latter seems to be the case, for the bridge at the left (indicated by the arrow) is definitely more stretched out than the other, and so must have originated from a

more proximal inversion. Our data likewise seem to disprove the presence of long inversions in this group of Tradescantia.

A crossover within an inversion should produce at anaphase I a dicentric chromatid and an acentric fragment. The latter, lacking a centromere, possesses no means of being assured regular distribution. It remains free in the cytoplasm, usually in the vicinity of the bridge (Plate 1, figures G, J), but occasionally at the edge of the spindle (Plate 1, figure E). It seems significant and worthy of mention that more than half of the cells bearing bridges lacked the fragment (Plate 1, figure D, I). This phenomenon has been encountered by both SAX (1937b) and DARLINGTON (1937) in Tradescantia, and by STEBBINS and ELLERTON (1939) in Paeonia, although not in such a high percentage of cells. These investigators postulate the presence of small sub-terminal inversions, and this is apparently true in the present case, for the bridges are approximately double the length of a chromosome arm, and with a diameter comparable to that of a normal arm. It would seem that some underlying cause must be responsible for the disappearance of the fragment, for too overwhelming an amount of evidence has accumulated disproving terminal fusion of chromosomes under natural conditions. Without doubt some of the fragments are too small to be readily seen (Plate 1, figure E), while others are obscured by the other members of the complement. Fragments of all sizes have been observed, grading from those longer than a chromosome arm to fragments that approach the limits of microscopic resolution.

DESCRIPTION OF PLATE 1

Photographs of aceto-carmine smear preparations of meiotic configurations in Tradescantia. Ca. 1000×.

FIGURE A.—Meiotic metaphase. All chiasmata at terminal loci.

FIGURE B.—Meiotic metaphase. High interstitial chiasma frequency.

FIGURE C.—Meiotic metaphase. High interstitial chiasma frequency. Pair of univalents showing.

FIGURE D.—Meiotic anaphase. Inversion bridge minus the usual acentric fragment.

FIGURE E.—Meiotic anaphase. Inversion bridge. Tiny fragment indicated by arrow.

FIGURE F.—Meiotic anaphase. Inversion bridge with fragment attached to end of normal arm. Univalent dividing precociously.

FIGURE G.—Meiotic anaphase. Two inversion bridges of different lengths. Fragment from the left bridge has not been released as yet.

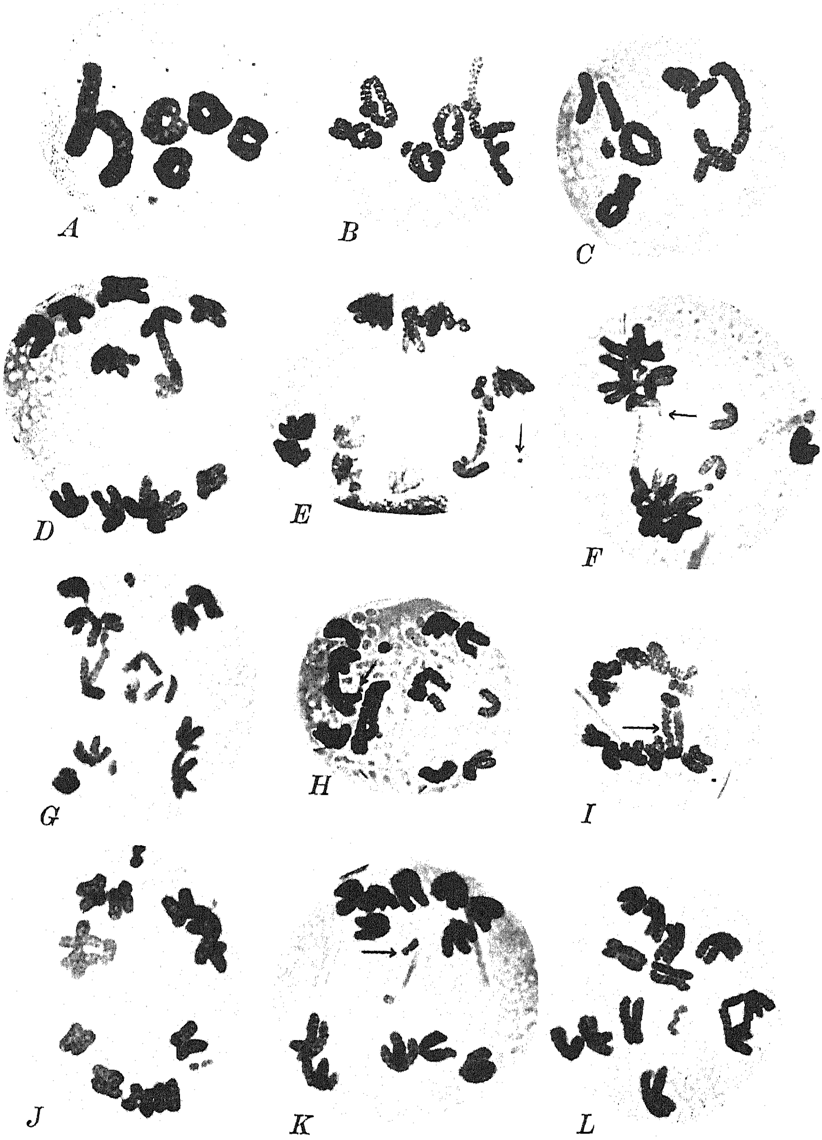
FIGURE H.—Meiotic anaphase. Three inversion bridges. Circular fragment visible (see text for explanation). Other fragment indicated by arrow.

FIGURE I.—Meiotic anaphase. Double inversion bridge. See text for explanation.

FIGURE J.—Meiotic anaphase from *T. paludosa*. Short inversion bridge and large fragment.

FIGURE K.—Meiotic anaphase from "Harvard" hybrid. Inversion bridge in the normal centric fragment. Acentric fragment indicated by arrow. Non-disjunction has resulted in a 5–7 distribution.

FIGURE L.—Meiotic anaphase from "Harvard" hybrid. Interstitial chiasma has delayed disjunction of the centric fragment.


A
B
C
D
E
F
G
H
I
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K
L

The work of McCLINTOCK (1938) offers a likely explanation for this phenomenon. In numerous instances, she has likewise failed to find the fragment free in the cytoplasm. However, in occasional cells she has detected the fragment attached to the end of the normal chromatid by means of a chromatid thread, and by which it was pulled poleward to be included in the telophase nucleus, in which case it would probably be lost in the cytoplasm at the second meiotic division. McCLINTOCK has postulated the probable occurrence of a chiasma between the inversion and the end of the chromosome, this chiasma tying the fragment to the normal arm. DARLINGTON (1937) states that in Tradescantia the fragment is "attracted" to the end of the sister chromatid, but this seems unlikely.

With considerable terminal association present in Tradescantia, the absence of the fragment can be easily explained in some instances by the terminal attachment of fragment and normal arm, and such configurations (Plate 1, figures F, K) have been frequently encountered, although the nature of the union could not be detected. The fragments are indicated by arrows. Furthermore, as stated above, fragments so small as to be almost at the limits of resolution have been found in some cells. Union of such fragments with the end of a normal chromatid would probably allow the acentric portion to pass to the pole unnoticed, its minuteness not appreciably lengthening the normal arm of the chromosome. The possibility of a chiasma distal to the inversion or inversions giving rise to the extremely small fragments seems remote since the pairing length would undoubtedly be limited, but no other explanation is at hand unless a matrical binding of some sort is postulated.

Numerous fragments were visible and, on measurement, showed considerable variation in length as well as in diameter. It should be stated that the use of diameter and length measurements of fragment size is not a dependable method for distinguishing one fragment from another, and thereby estimating the number of inversions present in a particular plant. The degree of contraction of the fragments is extremely variable, some remaining quite stretched out as indicated by the width between adjacent coils, while others round into spheres of chromatin. This is especially true when using acetic acid-alcohol fixatives. It is possible therefore only to distinguish between fragments of distinct size differences.

All evidence to date points to the fact that the size of the fragment from any one inversion is constant in length no matter at what locus within the inversion the crossover occurs. With this in mind, one is led to the conclusion that numerous small inversions have arisen throughout the interstitial regions of the chromosome arms, particularly in the sub-terminal regions. This is borne out by the high frequency of bridges with small fragments or no fragments at all. Small sub-terminal inversions

would be expected to produce crossovers very infrequently, but with numerous inversions of this kind, the total amount of crossing over within them would approximate the figure which might be expected from a much longer inversion. The infrequent appearance of long fragments rules out the presence of a long inversion in the regions of most frequent crossing over. Longer fragments do appear more often in those plants with a higher chiasma frequency, but this has been interpreted as resulting from an occasional crossover in a short inversion close to the centromere. The infrequent occurrence of a double bridge in a single arm (Plate 1, figure I) also militates against the presence of any long inversions.

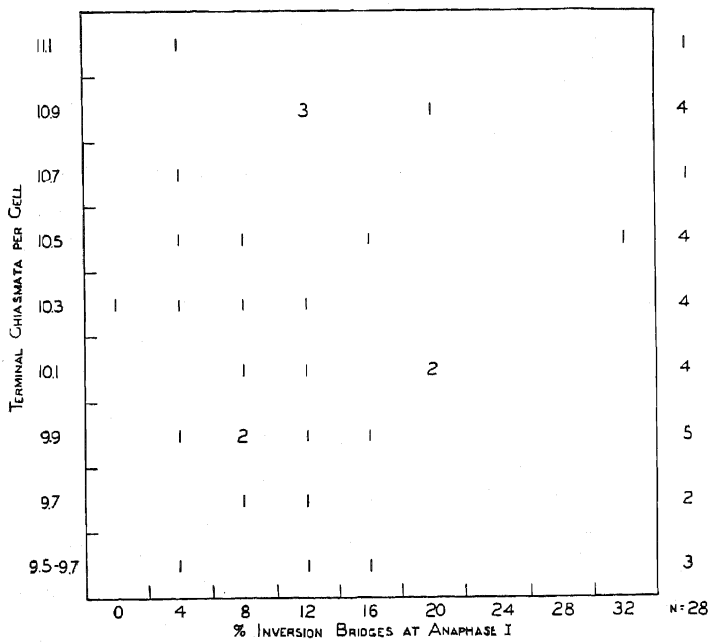

FIGURE 1.—Correlation between the frequency of terminal chiasmata per cell and the percentage of inversion bridges in the plants of the hybrid population. $r=.07\pm.18$.

The circular fragment found on one of the cells (Plate 1, figure H) is of interest. It could arise only from 2-strand double crossovers in adjacent (figure 4) or overlapping inversions (figure 5). This would release a circular acentric fragment as well as the usual straight acentric fragment (indicated by the arrow), which can be faintly seen in this figure attached to the lower normal arm of the extreme left-hand bridge.

If the sub-terminal and interstitial regions of the chromosome arms possess much inversion hybridity, and if the inversions are short and numerous rather than few and of random length, an additional inference can be made to the effect that an increase in crossing over in the total num-

ber of bivalents should be closely correlated with an increase in the percentage of cells showing inversion bridges. First it may be pointed out that no relationship exists between the number of terminal chiasmata and the number of interstitial chiasmata per cell, for if plotted against each other, a correlation coefficient approaching zero ($r = .043 \pm .19$) is obtained. A similar low value is obtained when the number of terminal chiasmata per cell is plotted against the percentage of inversion bridges (figure I). It may be concluded from this that the number of terminal chiasmata

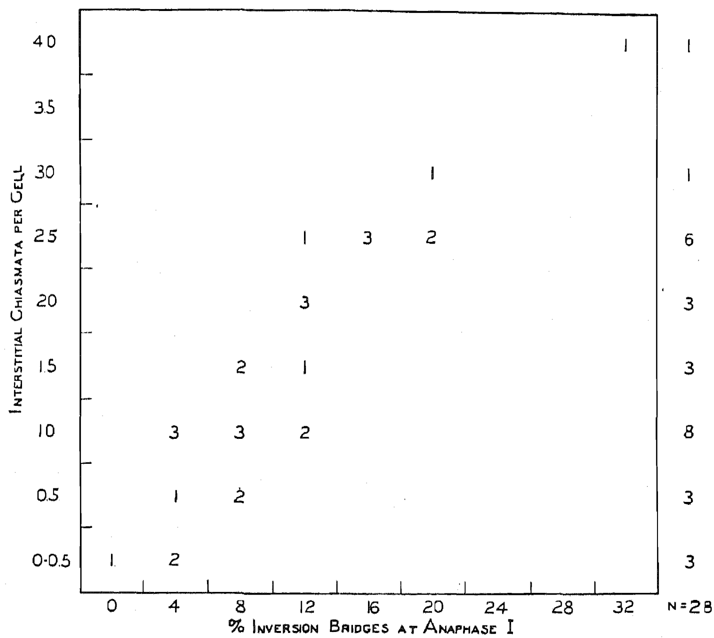

FIGURE 2.—Correlation between the frequency of interstitial chiasmata per cell and the percentage of inversion bridges in the plants of the hybrid population.

per cell approaches a value more or less constant for the genus regardless of the structural differences found in the interstitial regions of the chromosome arms, with such variation as might be expected from plant to plant.

If, on the other hand, the number of interstitial chiasmata per cell is plotted against the percentage of bridges, a high correlation ($r = .93 \pm .031$) is found (figure 2). This almost linear relationship indicates that the structural differences are equally numerous and probably quite similar in all the plants of the hybrid population, their ultimate expression being wholly dependent upon the particular chiasma frequency characteristic of the plant in question.

The occurrence of univalents in both the Oak Hill plants and in the

hybrid population was frequent (Plate 1, figure C, F). From what few observations were made as to the distribution and behavior of the univalents, they seemed to follow no regular course, although a good portion of them apparently reach either one pole or the other. Figure K and L (Plate 1) are cells from another plant, but they illustrate the unequal distribution of the chromosomes resulting from univalent formation. Rarely, the univalents were seen to divide equationally after the other paired chromosomes had passed to the poles (Plate 1, figure F).

The percentage of univalents varied greatly, among the fifteen plants examined for this irregularity, their frequencies ranging from zero to 30 percent (table 1). Since univalents result from an absence of chiasmata at metaphase, or from a premature separation of paired homologues, we should expect to find a fair negative correlation between chiasma frequency and the percentage of univalents. No such simple relationship is indicated by our data. STEBBINS (1938) has likewise pointed this out in Paeonia. MATHER (1936) has made it clear that in some plants crossing over in one bivalent is not entirely independent of crossing over in other bivalents in the same nucleus. Unfortunately, the individual frequencies for each bivalent cannot be obtained because of their close morphological similarity, but there does seem to be a tendency to maintain a certain level of chiasma formation, indicating that an increase occurs in the remaining bivalents if one of their number is unpaired. In the Oak Hill plants where univalent frequencies are high, it would seem that structural hybridity is the immediate cause of the unpaired condition. If, however, a comparison of these plants is made with such of the hybrids as plants 302 and 308 where bridge frequencies are high and no univalents are found in any of the cells, the more likely explanation is that a physiological incompatibility due to gene unbalance expresses itself by lowering the chiasma frequency, and that structural differences play but a secondary part in univalent formation. This would not apply to plants 42 and 105 where high chiasma frequencies are still accompanied by frequent univalents.

Physiological factors contributing to unbalance are likewise indicated in the sterility counts (table 1). In general, an increase in sterility follows an increase in the percentage of meiotic abnormalities, but the degree of sterility was always higher than could be accounted for on the basis of observed irregularities. Presumably certain chromosome combinations produce a gene unbalance as ANDERSON and SAX (1936) have suggested, these combinations being lethal in the haploid state. It is possible that undetectable interchanges occur in the interstitial regions giving rise to duplication and deficiency when in certain combination. The rôle of environmental fluctuations are known to affect pollen sterility to a marked degree,

even in pure species of Tradescantia (ANDERSON and SAX 1936), and in hybrid plants where physiological unbalance is undoubtedly present to some extent, environmental factors are probably even more effective in causing meiotic upsets than in the more stable types. Pollen fertility of Oak Hill 5 and 13 was high, being 79 percent and 83 percent respectively. This is a good deal higher than the fertility of the majority of the species-hybrids reported by ANDERSON and SAX (1936), and is quite possibly an indication that Oak Hill plants are not strictly F_1 hybrids, but that some backcrossing to the parent plants has increased the fertility.

Experimental data

The direct influence of the environment on chiasma frequency is indicated by two sets of data collected from plants 105 and 308 at different times (table 1). The variations are slight, but significant, since a correlated deviation is also seen in the percentage of inversion bridges. Within a physiological range, warmer temperatures seem to favor chiasma formation, particularly in the interstitial regions of the chromosome arms. To test this under controlled conditions, plants of a clonal line derived from a plant of known high bridge frequency ("Harvard" hybrid of unknown origin) were placed in a warm chamber (27°C) and in a cool greenhouse (12–15°C), and the meiotic behavior was compared with data collected from the same plants grown at the normal greenhouse temperatures (19–22°C). The data are given in table 2. The high percentage of univalents is due to the presence of a segmental interchange involving a short terminal segment, as shown by the formation of chains and rings of four chromosomes. Low temperatures obviously hinder crossing over, and in this interchange hybrid, the number of trivalents and quadrivalents is greatly reduced. It is evident that while physiologically favorable temperatures cannot completely overcome the lack of pairing conditioned by structural hybridity, such temperatures have an influential effect on pairing factors such that the interstitial regions are given greater opportunity to crossover. In the "Harvard" hybrid, as in the hybrid population, this

TABLE 2

Data on Harvard hybrid grown at different temperatures.

TEMP.	n	INTERSTITIAL XTA/CELL	TERMINAL XTA/CELL	PER CENT UNIVALENTS	PER CENT BRIDGES
12–15°C	70	1.6	6.07	78.5	17.5
19–22°	90	2.88	7.7	54.	26.1
27°		3.88	7.62	53.	47.8

increased crossover frequency in interstitial regions results in a marked increase in the bridge frequency.

The behavior of the so-called normal centric fragment found in Tradescantia is of interest. Although their origin is in doubt, the fragments are undoubtedly homologous with portions of the major chromosomes, for pairing with them does occur (DARLINGTON 1937). Such pairing has been occasionally seen in our material. All of the fragments found in this genus are not homologous with each other, nor are they morphologically alike. WHITAKER (1936) states that the kind found in *T. paludosa* Anders. and Woodson has a terminal centromere, and our observations bear this out (Plate 1, figure J). Figures K and L (Plate 1) taken from the "Harvard" hybrid show clearly in the bridge complexes that the fragment here has a medianly placed centromere. Interstitial chiasmata were formed in this small chromosome (Plate 1, figure L), interfering with its normal anaphase movement in such a way that it lags behind the major chromosomes, whereas it usually divides along with the others (Plate 1, figures G, J). The fragment, like the major chromosomes, possesses an inversion, and, rarely, forms an inversion bridge (Plate 1, figure K). The acentric fragment is being pulled poleward by its attachment to the arm of the upper normal sister chromatid (fragment indicated by arrow).

DISCUSSION

Meiosis in Tradescantia

Metaphase configurations in this genus are chiefly seen as ring and rod bivalents, with approximately 80 percent of all associations at terminal loci. Since pre-metaphase behavior cannot be studied favorably because of the difficulty of obtaining good preparations, earlier behavior must be deduced from metaphase configurations.

Pairing in Tradescantia is initiated at the distal ends of the chromosome arms, and proceeds from there in proximal direction. The reasons for this are not well known. WHITAKER (1936) suggests that strong polarization favors the initiation of distal pairing. However, other organisms in which pairing is initiated at the centromere are similarly polarized, so we can conclude that polarization alone is not the determining factor. It is quite possible that structural heterozygosity, due to inversions and internal non-homologies, is so great that pairing is delayed interstitially, permitting the relatively free homologous ends to come into paired alignment first. The evidence for distal pairing is both direct and indirect. Indirectly, the frequent occurrence of proximal interlocking of bivalents (ANDERSON and SAX 1936), the pairing behavior of fragment chromosomes, and the variation in the number of interstitial chiasmata as contrasted with the relative constancy in the number of terminal chiasmata are best interpreted by

assuming an early pairing of ends which permits terminal association, but which need not necessarily extend into interstitial regions. Direct observations have been made by NEBEL and RUTTLE (1936) revealing that, except for the occasional pairing of ends, very little homologous union has occurred. A strikingly parallel situation is found in Paeonia (STEBBINS and ELLERTON 1939).

It may be argued that this prevalence of termial chiasmata is a function of a high degree of terminalization as it is in some plants as Campanula. DARLINGTON (1929) has made diplotene observations indicating some movement of chiasmata toward the ends of the chromosome arms. The type of coiling in Tradescantia (SAX and HUMPHREY 1934) would seem to invalidate DARLINGTON'S observations, for no reversals in the direction of coiling were to be found as would be expected in 50 percent of the cases in which a chiasma had been formed, and was then terminalized. This datum would itself be invalidated were coiling initiated after the occurrence of crossing over, as it might well be. Two other arguments might be advanced. It may seem logical to postulate that the frequency of crossing over is similar in all of the plants and that the variation in the number of interstitial chiasmata per cell is but an expression of a variation in the degree of terminalization, that is, terminalization may be partial or complete depending on the time at which metaphase came in, whether early or late. Or again, the degree of terminalization may be looked upon as a function of structural heterozygosity, since the more heterozygous a plant is for inversions, the greater would be the obstruction to the terminalization of any proximal chiasmata which had been formed previously. Two bits of evidence, however, seem to leave little doubt that the amount of crossing over, and, hence, the chiasma frequency, is genetically determined. Firstly, the high correlation between interstitial chiasmata and bridge percentage (figure 2), and secondly, the correlation of temperature with an increase in chiasmata and bridge percentage point indubitably to a variation in crossing over and not to a variation in the degree of terminalization or a difference in structural heterozygosity. So while we recognize that some terminalization must take place since all chiasmata must necessarily have their origin interstitially, the movement necessary to reach the end of an arm is slight, and affects only those chiasmata lying in extreme distal portions. MATHER'S (1940) recent data are of interest in this respect, for he shows that the movement of chiasmata is apparently an all-or-none reaction, especially as regards interstitial chiasmata.

In order to evaluate the significance of chiasma localization in structural heterozygotes, it will be well to consider briefly how crossing over in Tradescantia is controlled. This control is exercised through at least three factors: (1) environment, (2) genetic factors, and (3) structural changes.

The first two are presumably operative in all organisms, while the influence of the last is dependent upon the kind and degree of structural differentiation present at any one time. All three factors exert an active control in Tradescantia.

The effect of environmental factors in this genus has been adequately demonstrated and discussed here and elsewhere (SAX 1937a). The evidence we have obtained in this instance is in partial accord with WHITE'S (1934) observations in the Acridiidae, where he showed that at the extremes of temperature the chiasma frequency showed significant increases. Our ob-observations in Tradescantia (table 2) differ in that at the low temperatures a considerable decrease in chiasma frequency, and a large increase in univalent formation, took place. Other evidence (SWANSON unpub.) seems to show that above 30°C a similar lowering of chiasma frequency is to be found, thus giving a unimodal curve instead of one with two maxima as WHITE obtains, and as PLOUGH (cf. WHITE 1934) has demonstrated for crossing over in Drosophila. STEBBINS (1938) postulates cooler temperatures as a factor in lowering the frequencies he observed in Paeonia. It may well be, as MATHER (1936b) contends, that any factor influencing crossing over will have its greatest effect in the vicinity of the centromere, and not at the ends of the chromosome arms. It is quite probable that this localization of effect may be due in part to the presence of heterochromatic portions in the regions involved (MATHER 1939), although no cytological proof of this in Tradescantia is at hand.

The influence of structural hybridity on crossing over is obvious. The introduction of any change in the linear arrangement of the genes reduces the possibilities of pairing, and may so interfere that by the time the ends have paired, for example, as in Tradescantia and the New World peonies, the interstitial regions will have passed through their division cycle, and will be in a condition prohibitive to crossing over. STURTEVANT and BEADLE (1938) show that in Drosophila, where pairing and crossing over originate at the centromere and proceed in a distal direction, an inversion exerts its greatest unfavorable influence—that is, it prevents the pairing of adjacent homologous portions—in regions distal to it. In Tradescantia, where pairing originates distally, the disruptive influences might be expected to be felt more in the regions proximal to the inverted portion. The fact that considerable structural differentiation is present in the hybrids, however, does not adequately explain the variation in chiasma frequency, as has been pointed out above. More likely, as an explanation is the fact that the individual chiasma frequencies are but the visible expression of certain combinations of genes governing meiotic behavior. Genic combinations favoring increased chiasma formation apparently so alter conditions at the time of crossing over that the regions of crossover frequency are

shifted toward the centromere, thus including portions of the chromosome arms which previously, in the Oak Hill plants, were undisturbed insofar as crossing over was concerned. The relatively inverted regions are provided with a greater opportunity for legitimate pairing, increasing the bridge frequency, and simultaneously increasing the number of interstitial chiasmata at metaphase, since all chiasmata within inversions as well as proximal to inversions would have difficulty in becoming terminalized. It seems clear, then, that an active genetic control of chiasma formation is present in Tradescantia, with structural hybridity playing a secondary rôle. PETO (1934) has previously postulated a genetic control of chiasma frequency in the Festuca-Lolium hybrids, while LAMM (1936) suggests that several basic genes, together with modifiers, could account for the difference in chiasma frequency observed in inbred rye.

Some difficulty is experienced in attempting to visualize clearly the exact manner in which the genetic control of meiosis is exercised. MATHER (1936) has shown in Drosophila that a temporal sequence in chiasma formation is present, the first formed chiasma being adjacent to the centromere, and then the succeeding chiasmata being formed distal to it. The centromere seems to be the determining mechanism in this case. In Tradescantia, with distal pairing and evidence of incomplete synapsis (NEBEL and RUTTLE 1936), it seems probable that a variation exists in the total paired length of the chromosomes at the time of crossing over. Possibly a time-limit to pairing is in effect. Since crossing over can only occur between paired homologues, the amount of crossing over, and hence chiasma formation, reflects the total length of the paired portions. In plants with a low chiasma frequency, the amount of pairing just reaches the threshold value necessary for sufficient chiasma formation to effect a regular meiotic distribution of the chromosomes.

It is evident that with chiasmata localized at the homologous distal ends, few meiotic irregularities appear. This is generally the case in the hybrid plants with a low chiasma frequency. On the basis of meiotic behavior of F_1 hybrids, ANDERSON and SAX (1936) have stated that there is little evidence of structural change in the complements of the different species of Tradescantia. From what we know now, this meiotic regularity in the first generation is to be expected, since the F_1 plants usually possess a lower chiasma frequency than the parental plants, and inversions would be even less in evidence than in the parents. This is indicated by data on inversion bridges in *T. paludosa* Anders. and Woodson, and an F_1 hybrid with *T. paludosa* as one of the parents (SAX 1937b, table 2, p. 526). Speciation, as judged from such observations, is seemingly a matter of gene differentiation alone, and it can be inferred that an interpretation of species relationships in Tradescantia, and possibly in other genera, based on

chiasma frequencies and meiotic irregularities in the F_1 generation may be misleading. When this is true, the F_2 progeny must be utilized to reveal the correct status of interspecific relationships insofar as they may be determined cytologically.

Inversion hybridity

The occurrence of inversions both in plants and in animals has been well established by many investigators: MUNTZING (Crepis, 1934), RICHARDSON (Lilium, 1936), DARLINGTON (Orthoptera, 1936), DARLINGTON and GAIRDNER (Campanula, 1937), STEBBINS (1938) and STEBBINS and ELLERTON (1939) in Paeonia, and DOBZHANSKY (1937) and others in Drosophila. It is now taken for granted that most natural populations will reveal inversions, this being particularly true in clonal lines, and in cross-fertilized organisms. In the former category, there is apt to be a gradual accumulation of structural changes, none of which *per se* will have any consequence other than to initiate possible changes due to position effect. In the latter, the frequency of inversions will vary widely depending upon the rate of occurrence, the probability of becoming established, the rate of elimination, and numerous other factors, none of which are sufficiently well understood at the moment to be evaluated.

The evolutionary significance of inversions and their rôle in the process of speciation has received the special attention of DOBZHANSKY and others (DOBZHANSKY 1937, DOBZHANSKY and STURTEVANT 1938), working on *Drosophila pseudoobscura*. The third chromosome of this species has provided a wealth of material, and by means of the salivary technique, it has been possible to establish the probable race history of *D. pseudoobscura* on the Pacific Coast. The inversion, as pointed out by DOBZHANSKY (1937), becomes a focus of discontinuity, and an opportunity for incipient speciation is at hand since within this gene sequence mutations can arise which, on the basis of random chance, will be unlike any other mutations. The differentiation is thereby intensified. Favorable gene combinations included in the inversion will aid in its establishment, and possibly in the establishment of a new variant. That this, however, is a long time process is indicated by the fact that with the great variability of inversions in *D. pseudoobscura*, the species morphologically is remarkably uniform and has very little recognizable diversity of external form (DOBZHANSKY and STURTEVANT 1938).

The fate of an inversion, or a group of inversions, is dependent upon a number of factors. It is evident that a short inversion possesses a distinct advantage over longer ones with regard to survival value since, as a rule, the difficulty of pachytene pairing, and hence crossing over, is in inverse proportion to the length of the inversion. This does not hold for salivary

gland pairing, where HOOVER (1938) has shown that there is no correlation between length of inversion and the degree of synapsis, for all inversions, regardless of length, pair completely in about 80 percent of all cells. No data exist comparing salivary and pachytene pairing, but it would seem that pairing time would be the limiting factor at pachytene but not so in the salivary gland cells. The advantage possessed by any particular inversion in maintaining itself free from crossing over is doubled if another inversion lies in the immediate vicinity. Chiasma localization is of equal importance, for if crossing over is restricted to homologous uninverted regions, inversions are similarly protected from elimination. Single crossovers are eliminated (STURTEVANT and BEADLE 1936), and only two and three strand double crossovers are effective in causing recombination within an inversion. This demands a comparatively long inverted section. STURTEVANT and MATHER (1938) adequately discuss advantageous heterozygosity in respect to favorable gene sequences and heterosis effects as it relates to inversions.

The occurrence of inversions in pure species of Tradescantia is apparently widespread. SAX (1937b) reported their frequency in four species; DARLINGTON (1937) reports inversions in diploid, triploid and tetraploid plants of *T. virginiana* L. and *T. bracteata* Small; GILES (unpub.) has located several in the complement of *T. rosea* Ventenat. A study of two plants of *T. paludosa* Anders. and Woodson from different localities gave bridge frequencies of 3 percent and 5.18 percent, with a considerable variation in the size of the fragment. These figures approximate SAX's (1937b) data on the same species. By arbitrarily dividing the chromosome arms into three parts, distal, median, and proximal, and then measuring the length of the fragment, an approximation can be reached as to number and location of the inversions. Ten measurements from one of the *T. paludosa* plants gave one fragment as long as $\frac{1}{3}$ of an arm, seven as long as $\frac{2}{3}$ of an arm, and three as long or longer than the single arm. A variation in the diameter of the fragment was also noticed, and several other bridges lacked the fragment. There are, then, at least four, and probably more, inversions in the chromosomes of this plant, but their apparent small size, and the localization of chiasmata, precludes a high frequency of crossing over within any one of the inversions. Figure J (Plate 1) illustrates the large fragment and short bridge found in this plant, but which was not encountered in the other plant of this species, although their chiasma frequencies were similar. Evidently the inversions in the two plants are not identical in location and length. Data from a triploid *T. bracteata* (SAX 1937b) and from triploids of the same hybrid population considered in this study (GILES unpub.) similarly indicate numerous inversions, and as would be expected, the percentage of bridges is generally higher than that found in

diploids since the effective pairing length is greater in triploids than in diploids.

Mention has been made of the fact that a goodly number of the cells bearing bridges lack the usual acentric fragment. This, together with the fact that numerous tiny fragments were found, points unquestionably to small sub-terminal inversions. It is perhaps difficult to understand why these small inverted sections should pair at all, but the type of pairing found in Tradescantia, and the sub-terminal location of the inversions, offers a likely explanation for this seeming anomaly. McClintock (1933) showed that small inversions as a rule pair non-homologously, or not at all. The torsional force of 2-by-2 association often forces the non-homologous regions into a paired condition. This is in Zea where pairing presumably is initiated at the centromere. With terminal pairing, it is not difficult to imagine that the relatively inverted sub-terminal regions of the arms, being capable of greater movement than an interstitial region, are freer to pair legitimately without the necessity of forming the customary loop, leaving the homologous strictly terminal regions unpaired. If the sub-terminal inversion is greater in length than the homologous portions distal to it, then the forces of homology at the time of initial pairing would be stronger in the vicinity of the inverted region than they would be in the neighborhood of the homologous distal portions resulting in straight inversion pairing rather than loop pairing (figure 3). The arm on the other side

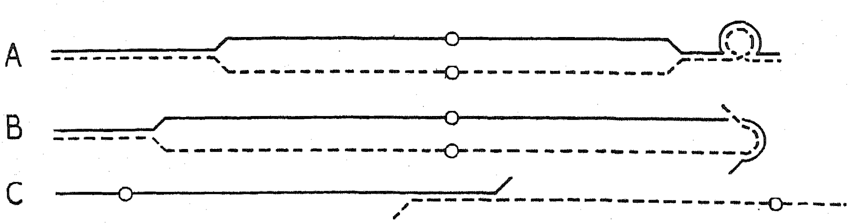

Figure 3.—Diagrammatic representation of how pairing can take place in short sub-terminal inversions where the inversion is longer than the uninverted region distal to it. Homologous chromosomes are shown as single rather than as double threads for the sake of clarity. *A* shows the conventional loop type of pachytene pairing. *B* and *C* show how inversion pairing can take place without the formation of a loop. See text for discussion.

of the centromere could move into position without hindrance, since polarization brings the arms relatively near to each other, or the bivalent could assume the form of a rod. The presence of a truly terminal inversion such as Kaufmann (1936) has found in Drosophila seems to be ruled out in Tradescantia by the fact that in nearly every plant, occasional cells showed 12 terminal chiasmata at metaphase, a condition not possible were such a heterozygous region present.

The presence of more than a single inversion in at least one of the chro-

mosome arms of this group of hybrid plants has been mentioned (figure H and I, Plate 1). These two anaphase configurations have probably arisen from double crossovers in two adjacent inversions, or in overlapping inversions. The possible configurations resulting from such double crossovers are schematically presented in figures 4 and 5. The figure showing both the circular fragment as well as the customary straight fragment (figure H, Plate 1) probably resulted from a 2-strand double crossover at loci 1 and 2 (figure 4, 5). 3-strand double crossovers (1, 3, in figure 4, and 1,4, in fig-

FIGURE 4.—Diagrammatic representation of crossing over in adjacent inversions. The loci at which crossing over takes place are designated by numbers. The chromatid configurations resulting from 2-, 3-, and 4-strand crossing over are given below.

ure 5) would give a double bridge configuration (Plate 1, figure I) with two acentric fragments. Unfortunately, neither fragment could be located although the unequal lengths of the two bridges leaves no doubt as to the correct interpretation. Configurations of the sort just mentioned have been previously described in Tulipa (UPCOTT 1937) where structural hybridity is extensive.

A configuration theoretically possible, but one which has not been observed, is that resulting from a 3-strand double crossover at loci 1 and 4 in adjacent inversions or at loci 1 and 3 in overlapping inversions. This type should occur with a frequency equal to that of either of the other two just described, but it is a type more easily overlooked at anaphase. It is evident from the diagrams that one of the free arms will contain a considerable portion of duplicated, and, in the case of adjacent inversions, of triplicated genic material. That is, the inverted portions of the chromosomes will be duplicated, and in an arm bearing two adjacent inversions, the re-

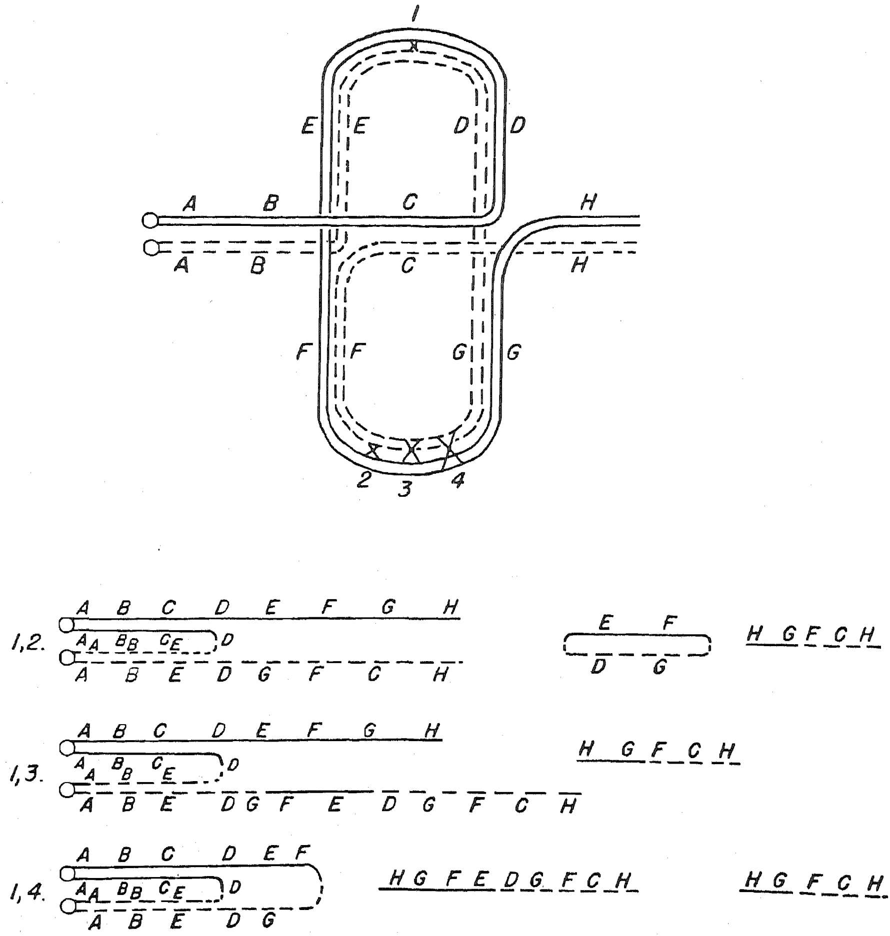

FIGURE 5.—Diagrammatic representation of crossing over in overlapping inversions. The loci at which crossing over takes place are designated by numbers. The chromatid configurations resulting from 2-, 3-, and 4-strand crossing over are given below.

gion between the inverted portions (region E in figure 4) will be in triplicate. In this manner we can see that inversions can act as a potential source of new chromosome types, although their ultimate survival and establishment is a moot question, since the addition of chromatin might readily lead to considerable unbalance and inviability.

A still further possibility for deriving new chromosome types may be demonstrated in figure 5. It is evident from this diagram that pairing in the *C* region presents an opportunity for crossing over. A single crossover in this region will produce two greatly altered chromatids. By following out the paired threads it will be seen that one chromatid will be extremely short, containing sections ABCH, whereas the other will be considerably lengthened and will consist of the regions ABEDGFCDEFGH provided

crossing over occurs at no other locus. Additional crossovers within the paired loops will greatly complicate the results. Starting with a chromosome possessing a median centromere, it is thus possible to derive two heterobrachial chromosomes, one having a sub-terminal centromere, and the other one sub-medianly placed. Although not known in the Virginiana Tradescantias, these chromosome types are found in other members of the genus, that is, *Tradescantia rosea*, as well as in other genera of the Commelinaceae (ANDERSON and SAX 1936), and while little is understood or known of the phylogenetic sequence within the family, the possibility of the derivation of new chromosome types through inversion crossovers should not be overlooked. There is likewise some evidence for the belief that the fragment chromosomes found commonly in Tradescantia might have a similar origin, for pairing of these fragments with major chromosomes has been seen to take place both at the terminal ends and in the region of the centromere. This is what would be expected if the interstitial regions were lost through some such mechanism as that postulated above, leaving a shortened arm on either side of the centromere. The relatively small amount of genic material added to the normal haploid complement by the retention of such a fragment would presumably contribute but little to meiotic irregularity or genic unbalance. This explanation of fragment origin is in better accord with the pairing relationships observed than is the alternative explanation of merely losing the distal portions of the chromosome arms, for with such an occurrence, no terminal pairing of the fragment would be possible. It may not be altogether coincidental that in Tulipa (UPCOTT 1937) and Fritillaria (FRANKEL 1937) where inversions are frequent and widespread, fragments are also common.

It has been previously mentioned that pollen sterility in the hybrid population is always higher than can be accounted for on the basis of observed irregularities at meiosis. Undoubtedly some mechanism contributing to genic unbalance in the haploid generation is responsible for the observed discrepancy. With numerous inversions present in Tradescantia, it seems not unlikely that some of the inversions may be of the included type (STURTEVANT 1938). This would be extremely difficult, if not impossible, to demonstrate cytologically in Tradescantia, but as STURTEVANT points out, structural heterozygote possessing chromosomes ABCDEFGH and AGCDEFBH—the latter the result of an inversion within an inversion—can pair and have crossing over take place in the CDEF region without bridge formation at anaphase, but that the chromatids involved will be both deficient and duplicated for certain genes. Pollen grains bearing these altered chromatids will be seen as empty grains at the time of maturity, since as GILES (unpub.) has shown, even small deficiencies are lethal before the microspore division has taken place.

From the data derived from the hybrid population we may infer (1) that the contributing species had become structurally differentiated in an independent fashion after their divergence from a common stock, and that this differentiation receives expression when the two genoms are brought together in the F_1 and later generations, or (2) that both parents possessed similar inversions which were established before divergence, and that genetic segregation had so shifted the location of crossing over that regions which up to this time had been more or less isolated were now undergoing a genetic shuffling. That the second is correct is indicated by the fact that all good species investigated to date have possessed inversions, but that the first inference is equally so is demonstrated by the considerable bridge frequency in a number of the plants in the hybrid population. In any event, the first has been shown to be dependent upon a favorable chiasma localization for visible expression at meiosis. It is very probable that in species which have become stabilized, an equilibrium is established to some extent between chiasma localization and inversions, such that inversions located in proximal regions can be carried on indefinitely without impairment to the species in question. Such plants as 101, 195, and 299, with a regular meiotic behavior, but with a low recombination rate as evidenced by a restriction of chiasma formation to the terminal regions, possess a selective advantage over those plants permitting crossing over to occur at random in the chromosome arms insofar as the maintenance of structural heterozygosity is concerned. Besides having a higher fertility, these plants also are in a better position to maintain favorable gene sequences (STURTEVANT and MATHER 1938) which might arise in the interstitial regions, for a low recombination rate will protect them from being broken up. This equilibrium, as we have seen, is upset in the F_2 generation due to genetic segregation and subsequent recombination of factors governing chiasma formation.

It is evident from figure 2 that there exists in the hybrid population an almost constant ratio between the number of interstitial chiasmata per cell and the percentage of inversion bridges, that is, for each increase of one interstitial chiasma there is an increase of approximately eight percent in the number of bridges. In other words, of every 12–15 chiasmata occurring at interstitial loci, one will be the result of a crossover within an inversion loop, and will express itself at anaphase as an inversion bridge. Figure 6 demonstrates this more clearly. Plants 325 and 50 depart markedly from this ratio, the former having a ratio of 6.2:1, and the latter a ratio of 27:1. This undoubtedly results from a greater structural heterozygosity in plant 325, and a greater structural homozygosity in plant 50 than is characteristic for the majority of the plants studied. For the group of plants as a whole, however, this ratio holds. The ratio, then, whether

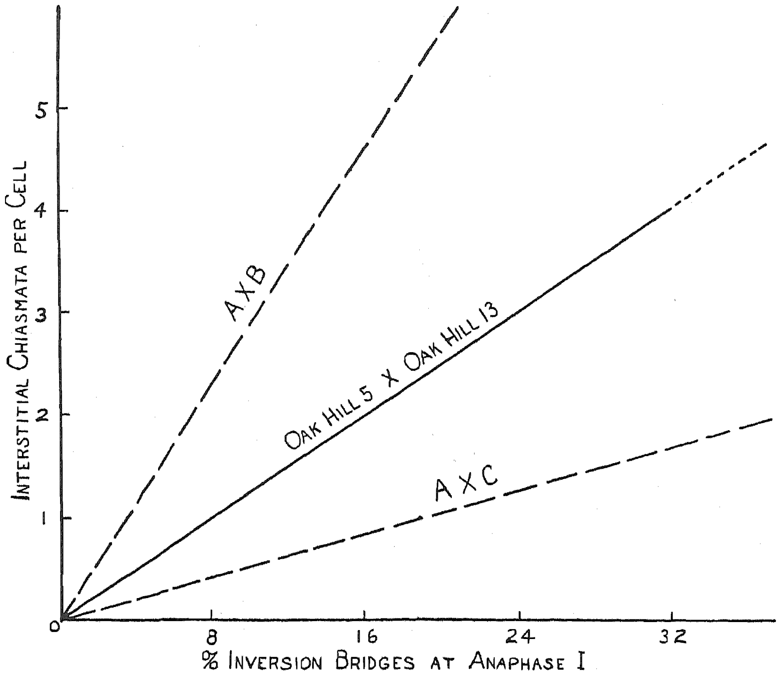

FIGURE 6.—Relation between interstitial chiasmata per cell and the percentage of inversion bridges in the offspring of the Oak Hill 5×Oak Hill 13 cross, as well as for two hypothetical crosses of Species A×B, and Species A×C. See text for discussion of the phylogenetic significance of such relationships.

high or low, has some phylogenetical significance, and it may possibly be of value in determining the degree of divergence of two species from a common stock. Thus, if species A were crossed on one hand with species B, and on the other with species C, and the ratios indicated by the curves in figure 6 were realized, one could conclude that, structurally, species B was more closely related to species A than was species C. The lower the ratio, the further the two species in question are separated, structurally, since a slight increase in the number of interstitial chiasmata per cell would raise the bridge percentage considerably. A high ratio would, on the other hand, indicate but little structural divergence, since with but few inversions, the chances of an interstitial chiasma occurring within an inversion loop would be greatly lessened since such loops would be of less frequent occurrence. It will be realized, of course, that this method of determining the relative inter-specific structural heterozygosity of a group of species has certain limitations: (1) the amount of intra-specific variation of this ratio must be rather thoroughly established before it can be of any value, (2) it can only be used where a large number of plants are available for cytological study since the ratio is determined for a group of plants, and not for single speci-

mens, and (3) it would probably be applicable only to such genera as Tradescantia, where inversions are small and numerous, and where factors governing meiotic pairing show segregation in the F_2 generation.

Of theoretical interest in Tradescantia is the fact that two conditions are found working in opposition to each other. These are (1) self-sterility, and (2) the presence of inversion with considerable chiasma localization. The first facilitates the recombination of genes responsible for variability, thus preventing genetic homozygosity; the second limits recombination, for unless two and three strand double crossovers occur, all inversions are either inherited as blocks of genes or are eliminated. HOOVER (1938) in a study of nine inversions of variable length in Drosophila finds that "cross over classes are completely suppressed within the limits of the inversion itself; furthermore, adjacent regions are strongly affected, the effect decreasing with distance from the aberration." The percentage of possible recombinations is therefore drastically lowered. In the hybrid population the barrier to recombination is somewhat overcome by increasing the limits within which crossing over can take place, thus including those regions which previously had enjoyed segmental isolation. The shuffling of gene blocks provides an opportunity for advantageous combinations to appear which, if provided with a means of perpetuation, will lead to variation and discontinuity within the group.

The presence of self-sterility in this genus undoubtedly accounts for the perpetuation of some of the structural heterozygosity. This phenomenon in Tradescantia agrees essentially with the oppositional factor hypothesis (ANDERSON and SAX 1934), in which it is postulated that pollen grains possessing *S1* and *S2* sterility genes will not send tubes down an *S1 S2* style, but will send tubes down an *S3 S4* style. If the sterility genes are located within an inversion, or in regions adjacent to an inversion, a mechanism is at hand to maintain such a structural heterozygosity from one generation to another. This, however, will account for only one inversion, and even stable species in this genus have more than this. Quite likely, in Tradescantia, is the possibility of a balanced lethal system such as is found in Rhoeo or Oenothera, with lethal genes being located in or adjacent to the inversions instead of being bound in with a segmental interchange complex. This hypothesis, however, awaits further analysis.

It has been pointed out previously (ANDERSON and SAX 1936) that within the *virginiana* group of Tradescantia the factors of importance in speciation have been genic differentiation, hybridization, and structural changes. These latter changes are fragmentation and segmental interchange. Evidence presented here, and by others (SAX 1937b, DARLINGTON 1937, GILES unpub.), makes it obvious that inversions must be looked upon as an equally if not more important evolutionary factor in this genus than those

just mentioned. Though it cannot be stated with certainty at present that inversions have played a major rôle in the differentiation of species, the great frequency of this type of structural change points to that fact and makes it quite probable. Considerable chiasma localization, the shortness of the inversions, and the factor of self-sterility have all undoubtedly favored the maintenance of inversions. Together with the possible position effects brought about by these rearrangements, it seems not improbable that inversions have provided the opportunity for genic differentiation to be preserved once it had taken place. However, more data concerning the degree of structural difference between the various species is needed to corroborate the evidence and to extend the information which has been obtained from the hybrid population described in this study, and hybridization between species is being carried out at present to achieve that end.

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SUMMARY

1. A cytological analysis has been made of two natural hybrids of Tradescantia and of 28 offspring resulting from reciprocal crosses between them. The analysis was concerned with chiasma, inversion bridge, and univalent frequencies, chiasma localization, and pollen sterility. It was found that few irregularities occurred at meiosis in the parental plants, but that a considerable variation was revealed in the hybrid population. The data are presented in table 1.

2. No correlation existed between the frequency terminal chiasmata and the percentage of inversion bridges, but a very high correlation was found between the frequency of interstitial chiasmata and bridge formation. This was interpreted as evidence that interstitial inversions are numerous in this genus, but that generally chiasma localization obscures this fact. With the segregation and subsequent recombination of genetic factors governing meiotic behavior, chiasma localization was interrupted, and crossing over occurred in regions that had previously been more or less isolated, thus revealing the inversions.

3. More than 50 percent of the cells bearing bridges failed to show the usual acentric fragment. This is discussed in some detail.

4. No correlation existed between chiasma frequency and the percentage of cells bearing univalents.

5. Sterility was observed to be always higher than could be accounted for on the basis of meiotic irregularities. Gene unbalance is suggested as a cause of a portion of the sterility, this unbalance possibly resulting from crossovers in included inversions.

6. Experimental data is presented showing that within a certain range, warmer temperatures favor increased chiasma formation particularly in the interstitial regions, with a correlated increase in bridge frequency.

7. Pairing in Tradescantia is initiated at the distal ends of the chromosomes, and proceeds in a proximal direction. Direct and indirect evidence to this effect is given.

8. The control of crossing over is discussed from environmental, structural, and genetical points of view. Genetical factors play a most important part in the determination of chiasma frequency and localization, with the other two factors playing minor rôles.

9. The evolutionary rôle of inversions is discussed. The widespread occurrence of inversions in this genus suggests that these aberrations have played an important part in speciation.

10. Double crossovers in adjacent and overlapping inversions are discussed and diagrammed. Their possible rôle in the production of new chromosomes is brought out.

11. An almost constant ratio exists between the number of interstitial chiasmata per cell and the percentage of bridges when considered for the whole group of offspring. The phylogenetical significance of this ratio is suggested, since in any group of F_2 plants derived from a species cross, it will determine the relative amount of structural divergence between the two species. Its limitations are also discussed.

12. Two factors, opposite in action, are found in Tradescantia. These are (1) self-sterility which facilitates recombination, and (2) inversions together with chiasma localization, which act as barriers to recombination. These barriers are somewhat overcome in the F_2 generation by the segregation and recombination of factors governing meiotic behavior.

13. It is pointed out that self-sterility genes, if located within, or adjacent to, inversions serve as mechanisms for the perpetuation of inversion heterozygosity.

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ON THE NATURE OF X-RAY INDUCED DELETIONS IN TRADESCANTIA CHROMOSOMES

CHARLES M. RICK
Harvard University, Cambridge, Massachusetts

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THAT deficiencies of chromatin account for many of the mutations resulting from X-rays has been well established by the work of DEMEREC, KAUFMANN and SUTTON (1939) and others in Drosophila as well as by that of STADLER (1932) and McCLINTOCK (1931) in Zea. The production of chromosome aberrations in Tradescantia by the application of X-rays has been thoroughly investigated by SAX (1940). His observations were limited mostly to dicentric and ring types which generally can be interpreted as inviable rearrangements due to gross deficiencies or mechanical difficulties encountered in subsequent mitoses. The relative proportion of viable rearrangements such as inversions and reciprocal translocations could be determined from such data. SAX (1938) has also noted the occurrence of small acentric fragments not involved in other chromosome rearrangements and has proposed that these and small inversions might be attributed to breaks in adjacent gyres induced by a single hit followed by fusion of broken ends into a new association. This theory has also been suggested by BUCK (1939). In view of the fact that such deficiencies may give rise to phenotypic effects and thus appear as mutations, the present investigation on the structure and origin of chromosome deletions was undertaken. A brief abstract of this work has already been published (RICK 1940).

MATERIALS AND METHODS

These studies were limited entirely to one clone of Tradescantia which is free of small centric fragments. This plant is a segregant from a natural cross between *T. canaliculata* Raf. and *T. humilis* Rose. Throughout these experiments inflorescences were subjected to unfiltered X-rays generated by a Coolidge tube with tungsten target operating at 10 ma. at a peak potential of 160 kv. on a line voltage of 115 v. Dosage was always varied by regulating intensity, that is, by regulating the distance from target to inflorescences according to the inverse square law.

Five to ten days subsequent to X-raying, anthers were smeared; preparations were fixed in alcohol-acetic and stained in aceto-carmine. The chromosomes were observed in metaphase and anaphase of the first mitotic division in the microspore.

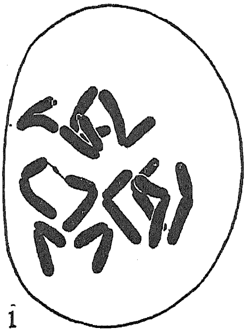
STRUCTURE AND ORIGIN OF DELETIONS

Except for a few rare instances of chromatid aberrations, which can be accounted for as spontaneous breaks (cf. GILES 1940), all rearrangements observed at anaphase and metaphase involved both sister chromatids at the same locus, and thus, as has been well demonstrated by RILEY (1936), MATHER (1937) and SAX and MATHER (1939), are accounted for by breaks induced when the chromosome was effectively single.

As all breaks affect sister chromatids alike, the deletions are always paired and usually in contact, although they may be separated (figures 2, 6, 7, 8 and 9). These deletions are free from other chromosomes excepting rare cases in which they seem to be attached to the ends of separated sister chromatids. Figure 4 exemplifies such terminal attachment, which might conceivably result from unequal interchange, one of the breaks of which occurred in a satellite. At anaphase they show little tendency to separate poleward but remain near the equator or drift to one side, apart from the more orderly centric chromatids.

Regardless of their size, acentric rod fragments bearing two normal chromosome ends are immediately identifiable by their appearance in the same cell with ring or dicentric chromosomes (figures 2, 3, 4, 6, 9). They constitute the exchange terminal deletions of figure 10. Acentric fragments produced independently of such configurations must represent either subtractions from the end of, or portions excised from the interstitial regions of, a chromosome arm, as seen in the simple terminal and simple interstitial deletions respectively of figure 10. Practically all critical evidence has demonstrated that broken ends of chromosomes very seldom remain unfused and the refusion which does occur is always between broken ends (GOODSPEED and UBER 1939). In view of this evidence, the simple terminal deletion should be rare and should appear rod-shaped. Fragments of U-shape, which result from fusion between two broken ends resulting from splitting of terminal deletions have been observed only very rarely. In accord with the above expressed view the simple interstitial deletion would be of ring shape.

The details of internal structure of metaphase and anaphase chromosomes are seldom visible in these preparations. Moreover, small centric ring chromosomes (figure 2) cannot be distinguished morphologically from small rod fragments (figure 3). Thus a morphological distinction between ring and rod fragments can be made only when the length of the chromosome portion deleted considerably exceeds the normal diameter of metaphase chromatids (figures 5 and 6). In a sample of 831 deletions from doses of 200 to 600r, 107 were sufficiently large to be classified definitely into 76 or 9.2 percent of ring shape and 31 or 3.6 percent of rod shape. Of the remaining 724, that is, the great majority of all chromosome deletions



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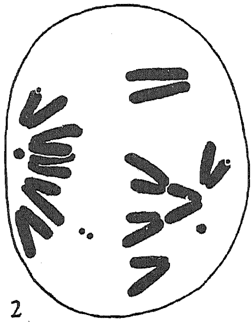

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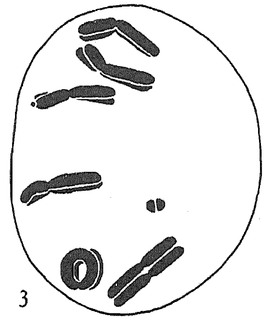

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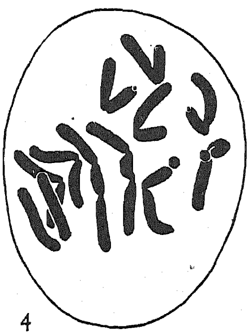

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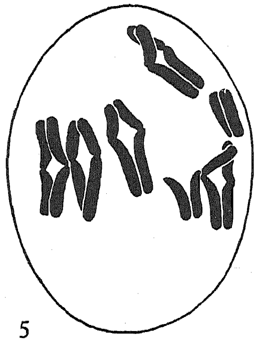

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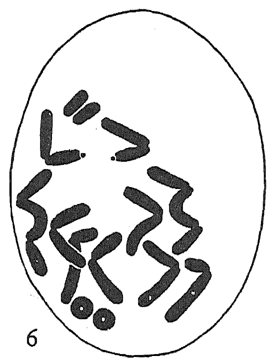

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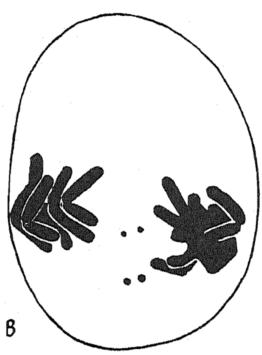

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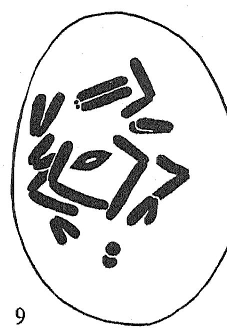

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Explanation of Figures 1–9

Camera lucida drawings of microspore division figures from smears prepared 5 to 10 days following X-ray radiation. Magnification about ×1000.

Figure 1.—Anaphase showing no aberrations. Both satellites are visible in typical position.

Figure 2.—Anaphase showing very small centric ring chromatids and correspondingly large rod (exchange-terminal) fragments in addition to a pair of simple deletions.

Figure 3.—Metaphase showing large centric ring chromosome and correspondingly small rod fragment.

Figure 4.—Early anaphase showing a pair of simple deletions apparently terminally attached, also dicentric chromatids with rod fragments.

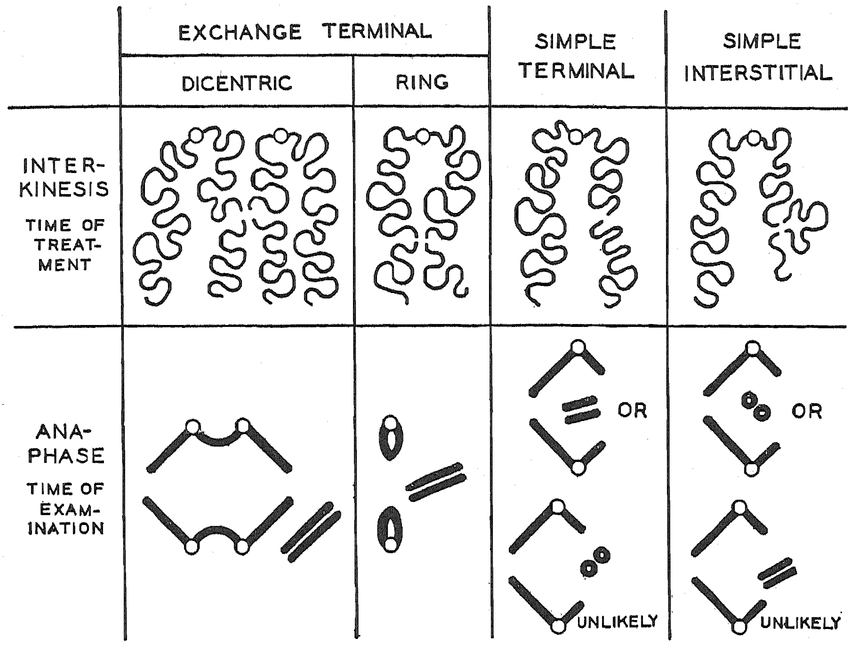

FIGURE 10.—Acentric fragments occurring as chromosome aberrations in Tradescantia.

produced, 71 percent might be expected to be of interstitial origin, if the same proportions occur as in the case of large fragments. Further consideration, however, indicates that this fraction must be much higher.

Where data are available, they indicate (table 1) that, when the frequency of large ring fragments is plotted against X-ray dose, an exponential curve is secured which may be taken as evidence that these are two-hit aberrations of interstitial origin. The designation, "hit," may be regarded as synonymous with ionization because it is the only likely X-ray effect concerned which is produced independently of wave-length. The writer (RICK unpublished) has found breaks in Tradescantia to be of approximately equal frequency at different wave-lengths of applied radiation. A similar finding is reported by EBERHARDT (1939) in Drosophila insofar as a change in phenotypic expression of the dominant allele of *cubitus inter-*

FIGURE 5.—Early anaphase showing a large simple terminal deletion resulting from a break close to the centromere.

FIGURE 6.—Anaphase showing a pair of large ring shaped simple interstitial deletions and deficient arms in addition to dicentric chromatids with fragments.

FIGURE 7.—Anaphase showing two pairs of simple deletions. Four satellites are detectable.

FIGURE 8.—Late anaphase showing two pairs of simple deletions.

FIGURE 9.—Anaphase showing centric ring chromosome, the rod fragment of which bears satellites. A pair of simple deletions is present. Abnormal arm length in chromatids suggests unequal reciprocal translocation.

TABLE 1

Frequency of large ring deletions following three successive doses of X-rays. Time: 8 minutes; smeared 5 days after radiation.

INTENSITY	DOSE	TOTAL CHROMOSOMES	LARGE RING DELETIONS	% RING DELETIONS	EXPECTATION FROM $(D/347)^{1.9}$
25r/min.	200r	5832	22	0.37	0.35
50r/min.	400r	1027	12	1.17	1.29
100r/min.	800r	600	30	5.0	4.7

ruptus is actually a measure of translocation. This is in close agreement with the report by MULLER (1938) that large interstitial deletions in Drosophila vary as the 3/2 power of dosage where radiation was evidently administered at the same intensity but for different lengths of time.

Similar data for large rod deletions are presented in table 2, where the linear relationship between X-ray dose and their frequency argues that they result from one hit and consequently are terminal.

TABLE 2

Frequency of large rod deletions following three successive doses of X-rays. Time: 4 minutes; smeared 5 days after radiation.

INTENSITY	DOSE	TOTAL CHROMOSOMES	LARGE ROD DELETIONS	% ROD* DELETIONS	EXPECTATION FROM $(D/1830)^{1.0}$
25r/min.	100r	5796	4	0.06	0.056
50r/min.	200r	5832	6	0.10	0.117
100r/min.	400r	5220	13	0.25	0.229

* These figures represent a mean of samples (slides) of different sizes and therefore are not exactly equal to the quotient of the two preceding columns.

The nature of the remaining small deletions must be determined by less direct means. In order that a terminal rod fragment may be detected as such, it must involve at least $\frac{2}{5}$ of the average arm of the Tradescantia chromosome. It follows that these result from breaks in the proximal $\frac{3}{5}$ of the arm. SAX and MATHER (1939) have demonstrated that 72 percent of all breaks yielding dicentric chromosomes occur in this region. If the distribution of breaks responsible for terminal deletions is of a similar nature, and there seems to be no reason to doubt this, terminal deletions should constitute only $\frac{31}{0.72 \times 831} \times 100$ or 5.2 percent of all deletions and a still smaller portion of the smaller deletions.

There is no great inequality in length of arm in the chromosome complement of the Tradescantia clone studied here as figures 1–9 will testify; at least calculations based on the assumption that all arms are of equal length should not be far from correct. Two chromosomes in the haploid set bear minute satellites on their shorter arms. Both satellites are detectable in figure 1 and figure 7. In a sample of nine slides from this study it was found that an average of 51.5 percent of the satellites are detectable. For each slide the number of cells in which two, one, and no satellites were observed approximated very well, as judged by the χ^2 test, the values expected from the expansion of $(p+q)^2$ where p = the proportion of all satellites observed for the particular slide and $q = 1 - p$. That there was no excess of cells with only one satellite visible argues that each satellite is equally detectable. This point in addition to the fact that the two arms bearing satellites are of equal length suggests a polyploid origin of these structures (cf. GATES 1939). Now if $\frac{1}{6}$ of the chromosome arms bear satellites and these satellites are detectable in about $\frac{1}{2}$ of the chromosomes, some estimate might be made of the proportion that terminal rod fragments constitute of all the smaller fragments. That they do constitute only a very small fraction is shown by the record of only two satellited fragments in some 1500 small fragments examined. It might be argued that for some reason satellites are less easily detected on rod fragments. Yet as RILEY (1936) points out, acentric fragments show the same developmental cycle as unaltered chromosomes. In the sample of nine slides, the proportion of 200 rod fragments of the exchange terminal type which bore satellites compares very well with values calculated on the assumption that there is equal likelihood of exchange occurring between any two chromosomes (cf. figure 9). Further, this may be taken as evidence that exchanges occur at random between chromosomes.

The relation between the frequency of small simple deletions and dose is given for one series in table 3. Large rod fragments detectable as such are not included here, but large ring fragments are included. Since the latter constitute only about 10 percent of the total, their frequency if different would not seriously affect the result.

In two other series approximating the same number of chromosomes but at different doses, the best fit was secured to curves having as exponents the values 1.80 and 1.62, the average being 1.67. The exponents of exponential curves best fitting exchange ring and dicentric breaks recorded in the same samples were, respectively, 1.94, 2.10, and 1.97, agreeing closely with values obtained by SAX (1939 and 1940). The average exponential curve for simple deletions would be approximated for the dosage range studied here if at the lowest dose 40 per cent were one-hit aberrations and 60 percent two-hit aberrations. Obviously terminal

deletions could not account for this proportion of one-hit deletions. The problem of accounting for this 40 percent will be considered further in the discussion.

Curves like this one representing the relation of a sum of one- and two-hit effects to dosage should approach linearity at very low doses and a parabolic form at much higher doses. Since the dosage range of 100 to 600r units utilized here represents the only part which can be analyzed satisfactorily with the method of scoring used, no test of these trends was possible. Confirmatory results have been published by FABERGÉ (1940) although differences in material, methods of scoring, etc. would not permit a direct comparison. Doses of 1320, 2640, and 3960r secured by varying the intensity, were applied and the method of scoring was merely to record the total number of pieces of chromosomes observed at meta-

TABLE 3

Frequency of small simple deletions following three successive X-ray doses. Time: 4 minutes; smeared 5 days after radiation.

INTENSITY	DOSE	TOTAL CHROMOSOMES	SIMPLE DELETIONS	% SIMPLE* DELETIONS	EXPECTATION FROM $(D/133)^{1.58}$
25r/min.	100r	5796	37	0.64	0.64
50r/min.	200r	5832	117	1.94	1.91
100r/min.	400r	5220	296	5.6	5.6

* These figures represent a mean of samples (slides) of different sizes and therefore are not directly equal to the quotient of the two preceding columns.

phase in the microspore division of *Tradescantia bracteata* Small 70 hours subsequent to X-ray treatment. If breakage and refusion behave at these high doses as they do at lower doses, these pieces should be chromosome aberrations; the only types of these that result in an increased number of chromosome pieces at metaphase are centric ring chromosomes with their rod fragments, and deletions, the latter comprising by far the greater part. Now, if the above interpretation is correct, at the high doses used by FABERGÉ aberrations should increase with the square of applied dosage. If the values obtained are plotted logarithmically with respect to dose and aberrations, the frequency of aberrations is found to vary as the dosage raised to the powers: 2.06, 2.08, 1.55 and 1.38, in four different series, although it is claimed by FABERGÉ that the values obtained do not deviate significantly from those expected if the relation were linear. The discrepancy between the first two and the last two of these values is rather large, but it might not be significant in view of the relatively small number of cells on which these four curves were established.

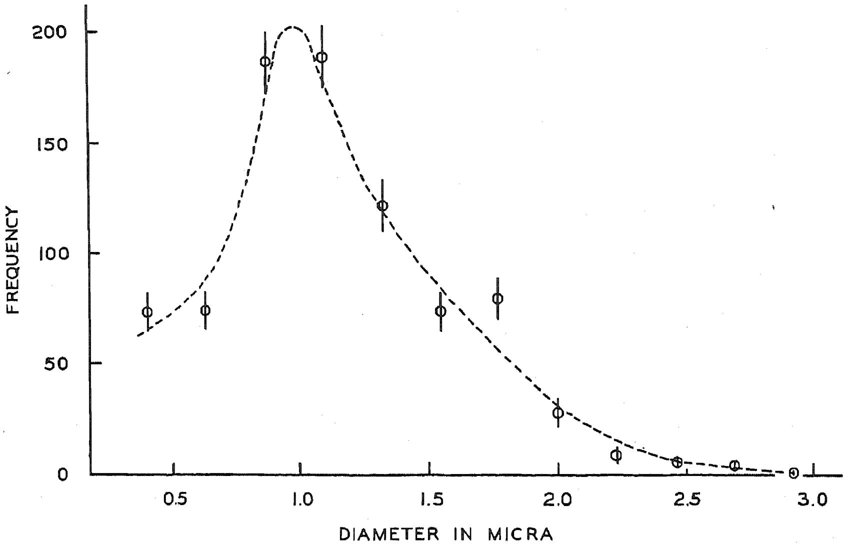

FIGURE 11.—Frequency distribution of deletion size. Vertical lines represent standard deviations of obtained values.

The mean value is 1.77, not much higher than the value obtained above. This result is still probably in keeping with the expected value, 2, for it is known that the division cycle in Tradescantia is notably retarded by heavy X-ray doses, so that even when the natural cycle is at a minimum, some one-hit chromatid aberrations would be expected 70 hours after treatment. These one-hit aberrations are of the simple deletion and single dicentric chromatid plus U-shaped fragment types which would increase the number of chromosome pieces observed.

The values observed by FABERGÉ at these high doses agree remarkably well with values calculated by extrapolation from data in the present experiment, although this might not be expected due to many sources of error such as differences in sensitivity of the material, and possible discrepancies in measurement of ionization which might exist between these experiments. This rough agreement certainly does not prove that the type of aberration at high doses is similar to that at low doses, but it is quite compatible with such an assumption.

Further evidence that simple deletions are chiefly two-hit chromosome aberrations and therefore probably interstitial is to be found in observations of spontaneous breaks. GILES (1940) noted the relative rarity of two-hit chromosome aberrations arising spontaneously and *a fortiori* noted the complete absence of simple deletions in any of his extensive studies.

The distribution of size was recorded for a sample of the deletions studied (figure 11). Since these simple deletions are paired and appear spherical, the diameter was secured by dividing by two the distance across a pair.

Deletions were measured only at metaphase and early anaphase in order to minimize variation due to the coiling cycle. If two breaks occur at random in any chromosome arm where breaks are evenly distributed throughout the arm, the relative frequency of deletions of a given length will be inversely proportional to that length (MULLER 1938). The frequency distribution expected on these grounds may be expressed as a straight line whose equation is $y=(-2N/l^2)x+2N/l$ where N = number of fragments and l = length of the arm. Limitation of breaks to any region, as has actually been demonstrated by SAX and MATHER (1939), would tend to increase the expectancy of the smaller deletions, again the increase varying inversely with size of the deletion. Thus, in spite of the fact that the smallest deletions of figure 2 would be most likely to escape observation, the sharp decrease in their numbers in comparison with those clustered around 1μ is probably real.

By similar reasoning, an estimate can be obtained for the proportion of excised deletions that should include the centromere when it is in median position—a reasonably accurate assumption for Tradescantia chromosomes. Under these conditions any deletion larger than half the length of the chromosome will include the centromere, and for smaller deletions the proportion which include the centromere should equal twice the ratio of the length of the deletion to the length of the chromosome. Deletions which include the centromere are, of course, the centric ring chromosomes produced by exchange breaks and those not including the centromere constitute almost all the deletions considered here. If the expected proportion of centric rings for each size is calculated from the data available in this study, a figure of 43 per cent is obtained. The actual proportion of centric rings in this sample is 16 percent.

Another deviation from expected values is to be found in the ratio of deletions to dicentric chromosomes. If breaks are distributed at random to the twelve chromosome arms, and this is supported by evidence encountered above, the probability of two breaks within one arm is 1/12 and that of breaks in two different chromosomes is 10/12, the ratio between the two types of events being 1/10 or 0.10. The proportion of deletions which is produced by two hits, that is, 60 percent at lowest doses, corresponds to the event of two breaks in one arm and the analogue to two breaks in two chromosomes is the dicentric chromosome. The actual ratios between two-hit deletions and dicentrics in three series are 1.20, 1.46, and 2.02, revealing a tremendous excess of deletions above the expected value. CATCHESIDE (1938) and BAUER, DEMEREC and KAUFMANN (1938) reported a similar excess in Drosophila, where the ratio of intra-arm inversions to inter-arm inversions and translocations was $\frac{1}{2}$ compared with the ratio $\frac{1}{4}$ expected on random distribution of breaks.

The reasons for the great discrepancies noted both in the frequency distribution of deletion size and in the ratio of deletions to other types of aberrations are to be sought in the spatial limitation imposed upon refusion of X-ray induced breaks. Other evidence that refusions must be localized and thus influenced by the spatial disposition of the chromosomes at time of treatment is given by BAUER, DEMEREC and KAUFMANN (1938) and SAX (1940). It is well established that relic coils persist through the resting stage and are still detectable at the ensuing prophase and it seems very likely that such coiling can account for these two discrepancies. The existence of relic coils should greatly enhance the opportunity for refusions within a chromosome arm and also for refusions between ends of deletions of certain restricted size classes and these are precisely the two effects noted. The size relation which the fragments of modal frequency in figure 2 bear to the whole chromosome is approximately what would be expected if there are 20–25 minor coils per chromosome as observed in Tradescantia in occasional favorable preparations here and also by SAX and SAX (1935). It should also be noted that during the resting stage considered, any coils which may exist are relic from anaphase chromosomes of the second meiotic division and that numbers of minor coils have been ascertained from anaphase chromosomes of somatic mitoses. Nevertheless, the coiling in both is known to be sufficiently similar to afford this rough comparison.

SAX (1940) has discovered that exchanges between arms of the same chromosome occur much more frequently than expected, if exchanges occur at random between all chromosome arms. Thus, the expectancy of centric rings might be expected to exceed the value of 43 percent calculated above. In spite of this effect, the opportunities for refusion between two breaks within one chromosome arm afforded by relic coiling must be of much greater moment than opportunities for refusion of breaks favored by the proximity of two arms as evidenced by the very low proportion of centric rings obtained.

RELATION OF DELETIONS TO OTHER ABERRATIONS

None of the records suggest a tendency toward either association or dissociation between simple deletions and the exchange breaks, that is, dicentric and ring chromosomes. Where a comparison was made with values expected if these aberrations are produced independently, as in table 4, there is no greater discrepancy than would be expected from random variation.

EFFECT OF TEMPERATURE ON X-RAY INDUCED DELETIONS

The temperature difference was maintained for a period from a few

TABLE 4

Association in the microspore of simple deletions and exchange breaks. 5 slides. 869 microspores. Dose: 400r.

		NUMBER OF SIMPLE DELETIONS							
		NONE 70.6%*		ONE 25.2%		TWO 3.8%		THREE 0.4%	
		OBS.	EXP.	OBS.	EXP.	OBS.	EXP.	OBS.	EXP.
Number of exchange breaks	None** 77.7%	461	478	176	170.5	25	25.6	3	2.6
	One 20.2%	144	124.5	40	44	8	6.5	0	0.7
(Ring and dicentric chromosomes)	Two 2.0%	9	12.2	3	4.3	0	0.3	0	0.1
	Three 0.1%	0	0.6	0	0.2	0	0	0	0

* These percentages are derived from the Poisson distribution of a total of 34.2 percent of simple deletions.

** These percentages are derived from the Poisson distribution of a total of 24.6 percent of exchange breaks.

d.f. = 6 $\chi^2 = 5.656$ p = 0.3–0.5

minutes before radiation to one hour after radiation by warm water and by ice water in cardboard cartons. Hot and cold lots were irradiated simultaneously. Further details of treatment and results are given in table 5.

TABLE 5

Frequency of aberrations induced by X-rays at 3° and 33°C. Intensity: 100r/minute. Time: 3 minutes. Dose: 300r.

TEMP.	RADIATION	TOTAL CHROMOSOMES	DELETIONS*			EXCHANGE BREAKS. RING AND DICENTRIC CHROMOSOMES		
			NO.	%**	RATIO	NO.	%**	RATIO
3°C.	300r	3894	203	5.26	2.85	132	3.37	3.62
33°C.	300r	2988	54	1.84	1	27	0.93	1
3°C.	none	2100	0	0	0	0	0	0

* Deletions include all of the simple type except large rod terminal deletions.

** These figures represent a mean of samples (slides) of different sizes and therefore are not exactly equal to the quotient of the two preceding columns.

Since SAX and ENZMANN (1939) have already demonstrated a much greater yield of two-hit and most of the one-hit aberrations at lower tem-

peratures and since FABERGÉ (1940) noted a similar effect in unclassified aberrations, these results do not seem unusual. Moreover, it is not deemed necessary to experiment further as the former have adequately demonstrated by varying temperature subsequent to radiation that the temperature effect is a secondary one; namely, that the same number of breaks are produced at either temperature, but that refusion is slower at low temperatures, allowing more opportunity for broken ends to move away from old positions and fuse into new associations.

DISCUSSION

From the evidence presented above it is obvious that certain factors promote exchanges of broken ends within the chromosome arm and favor the production of deletions of a certain size class. It also seems very likely that these factors include relic coiling and a spatial limitation to refusion of broken ends. One further point in this connection deserves consideration. SAX (1938) has discussed the consequences of breaks and refusions in adjacent gyres of coiled chromonemata and has indicated that if the coil were fairly compact it is equally likely that a ring fragment deleted in this fashion will encircle or be free of the remaining chromonema. SAX (1940) has also found that in chromatid aberrations fusions resulting in dicentric chromatids are twice as frequent as fusions resulting in reciprocal translocations, indicating that following an effective X-ray hit the broken ends tend to shrink away from the point of the break. Now, if the coiling during the resting stage is relaxed with certain loci in successive gyres closer to each other than others, the shrinkage movement following breaks would favor the formation of free deletions. Actually, no cases of deletions embracing metaphase chromosome arms were encountered. A deletion resulting from breaks in successive gyres would necessarily be small and, when locked around a chromosome arm at metaphase, might be undetectable. At anaphase, however, such a deletion might be expected to interfere with chromatid separation, but such a situation was never observed. On the other hand, deletions so locked might be slipped off the arms of separating chromatids and become terminally attached as in figure 4, yet most deletions are free from chromosome ends. Thus it seems most likely that relic coiling during the resting stage is of a very relaxed and irregular nature, which agrees with the condition insofar as it can be observed directly. It would also follow that small inversions, the formation of which is much favored by tight coiling, would occur relatively much less frequently in this relaxed coiling.

This study affords no criterion whereby one-hit aberrations can be distinguished from two-hit aberrations among the smaller simple deletions resulting from X-ray radiation. The curves obtained, which may be ac-

counted for by 40 percent of one-hit breaks at the lowest dose, include approximately 10 percent of the larger detectable rings which are all two-hit aberrations. Therefore, one-hit aberrations must constitute almost half of the remaining 90 percent of small deletions. Comparisons of size-frequency graphs of fragments produced at different doses show no consistent changes in form except for the greater proportion of larger visibly ring shaped fragments at higher doses, so that the one-hit types cannot comprise any particular size group of the smaller deletions. Might it not be possible that during the resting stage loops within an arm, including relic coils, might bring some portions of the chromosome into closer association than that existing between chromosomes—so close, in fact, that one hit could break both portions? The known spatial limits of the effect of one hit would not discount such an hypothesis, for SAX (1940) has demonstrated that an X-ray hit can break both sister chromatids at the same locus at a time when the split between chromatids can be visibly demonstrated. This point seems to be substantiated also by the work of STADLER and SPRAGUE (1936) who found that X-ray radiation of pollen yields a much lower rate of fractional deficiencies to entire deficiencies than does ultra-violet radiation. A direct cytological comparison of the effects of X-ray and ultra-violet radiation on chromosome aberrations has confirmed these genetic results (SWANSON 1940).

The independent work of several investigators has established beyond doubt the fact that in Drosophila the induced lethal mutation rate behaves as a linear function of the X-ray dosage, and thus the mutation measured is considered to be the result of a single X-ray hit (cf. TIMOFÉEFF-RESSOVSKY 1937). The slight deviation below the linear expectation noted at higher doses is quite close to the proportion of individuals expected to receive two or more mutations if a random distribution is assumed. A similar dosage-mutation rate relation has been revealed in plants where sufficiently investigated (STADLER 1930 and unpublished, GOODSELL 1930). Yet, as SAX (1940) and others have pointed out, if translocations and inversions, which most evidence indicates are the result of two independent hits, account for many of the observed mutations by position effect, then the exponent of the dosage-mutation rate curve should be greater than one. Moreover, if a large proportion of the deficiencies in Drosophila are two-hit aberrations, as found to be the case in the present experiments in Tradescantia, this should be reflected in an exponential trend of the dosage-lethal mutation rate curve.

The situation seems even more paradoxical in view of the finding of DEMEREC, KAUFMANN and SUTTON (1939) that translocations and inversions are directly associated with $\frac{1}{4}$, and deficiencies with $\frac{3}{4}$ of the lethals which display a phenotypic effect in the white-notch region, although

these lethals can scarcely be regarded as a random sample of sex-linked lethals. Further evidence bearing on this problem has been presented by TIMOFÉEFF-RESSOVSKY (1939) who reports that genetically determined inversions, translocations and large deletions which affect the X chromosome increase exponentially with increased X-ray dosage and at the highest dose tested, 6000r, are 40 percent as frequent as X chromosome lethals in the same material. The exact proportion of these chromosome aberrations which exert a lethal effect apparently is not known. Nevertheless, he estimated this proportion to be somewhat less than $\frac{1}{2}$, in consideration of the facts that all deletions measured are lethal in males; that the unbalance caused by segregation in a translocation heterozygote will not affect the measure of X-chromosome lethals; that some inversions and translocations do not produce a lethal position effect; and that some inversions and translocations occurring simultaneously with lethals in the X chromosome are not related in their breakage points to the locus of the lethals. He concluded therefore that the proportion of two-hit aberrations which exert a position effect is so small that even at the dose of 6000 r, this effect on the total lethal mutation rate would be statistically undetectable. This might be the case, if the proportion is very small, yet it scarcely seems possible if the proportion were 10 or 15 percent, which approximates the value given by TIMOFÉEFF-RESSOVSKY. As indicated above, the curve for lethal mutations does not show any such trend, but actually falls below the linear expectation at higher doses in accordance with the expectation of the chance occurrence of two or more mutations in the same chromosome. Certainly, if doses higher than 6000 r could be tested, the mutation rate should reflect the exponential rise of gross chromosome aberrations exerting position effect.

Elimination of gametes bearing two-hit aberrations cannot account for the difference because the method by which BAUER, DEMEREC and KAUFMANN (1938) found the exponential increase in gross aberrations was subject to the same elimination of gametes from treated males as might occur in the detection of lethals by the ClB technique. In view of the considerable sterility known to be induced by X-ray treatment, sterility of F_1 females bearing such aberrations might possibly account for the elimination. Obviously this could not be the case if TIMOFÉEFF-RESSOVSKY (1939) studied F_1 females in the genetic determination of frequency of aberrations. The mating techniques used were not explained in his paper. If this study was based on males, the above suggestion would then be adequate provided that sterility attending aberration is much higher in females than in males.

Since 60 percent of the deletions measured here are two-hit aberrations at the lowest doses and a much higher percentage at the higher doses, it

would follow that the mutations showing a linear relation to X-ray dosage could not be attributed to deficiencies of this sort. The possibility remains that very small one-hit deletions, undetectable due to the limits of resolving power of the microscope, and other minute changes in addition to point mutation are most responsible. This explanation gains support from the citations of MULLER (1938) and MULLER and MACKENZIE (1939) of the unpublished discovery of BELGOVSKY that the smallest deficiencies induced by X-rays in Drosophila vary according to the first power of the dose. Furthermore, DEMEREC (1939) suggested that small deficiencies comprising one to four bands of the salivary gland chromosomes of Drosophila might be one-hit phenomena and that larger deficiencies result from two independent hits because of the great excess of the former above the number expected if breaks were distributed at random. Chromosome deletions in Tradescantia as comparably small as one to four band deficiencies in Drosophila would surely be undetectably small.

DEMEREC (1937) advanced the hypothesis that each effective X-ray hit excises a small portion of the chromosome, and where two such hits occur within certain spatial limits, a refusion between broken ends in a new association would result in larger aberrations in addition to the small deficiencies. However this hypothesis seems to be contradicted by the findings of SOKOLOW (1937) and DEMEREC, KAUFMANN and SUTTON (1939) that few or none of the larger aberrations exhibit deficiencies at their points of breakage. Nor is any other hypothesis plausible which states that the frequency of mutations not associated with gross chromosome aberrations does not bear a linear relationship to dosage. At least in the case of lethals measured by the ClB technique, it seems most likely therefore, as TIMOFÉEFF-RESSOVSKY (1939) has proposed, that the gross chromosome aberrations having a mutational effect are so few in comparison with the total induced mutations that the dosage-frequency relations of the total rate are not statistically affected by the former at the doses ordinarily tested.

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SUMMARY

X-ray radiation of the post meiotic resting nucleus of Tradescantia microspores produces several types of simple deletions in addition to, and independently of, the deletions associated with the formation of ring and dicentric chromosomes. Of the simple deletions induced by X-ray doses of from 200 to 600r, 3.6 percent were visibly rod shaped, 9.2 percent ex-

hibited a detectable ring structure, and the remaining 87.2 percent were too small to be classified. The large rod shaped fragments are probably terminal deletions resulting from single hits (ionizations or ionization clusters) because their frequency is directly proportional to dosage. The large ring shaped deletions are considered to be two-hit aberrations of interstitial origin, since their frequency increases according to the square of the dosage. In the case of the small deletions, considerations of the distribution of breaks in the chromosome arm, and of the proportion of small deletions bearing satellites indicate that they are almost entirely of ring structure and of interstitial origin. The dosage-frequency relations of small deletions may best be accounted for by the hypothesis that at the lowest doses, that is, 100r and 150r, slightly more than one-half are two-hit aberrations and the remainder, one-hit aberrations.

An analysis of the distribution of the sizes of small deletions indicates that they are derived chiefly from breaks in adjacent gyres of the relic coils. Further evidence of the spatial limitation of refusion of broken ends and the consequent effects of relic coils is found in the frequency of small deletions relative to the frequency of ring and dicentric chromosomes. Thus two breaks are necessary for the formation of small deletions, and, as indicated above, these may result from either one or two hits.

X-ray radiation applied at lower temperatures results in a greater yield of chromosome deletions, as also of other aberrations, than does the same radiation at higher temperatures.

All of the chromosome alterations induced by X-rays which result in translocations, large inversions and deficiencies, and most of the visible small deletions in Tradescantia are produced by two independent hits and thus show an exponential increase with increased dosage. The same seems to be the case in Drosophila. Since it is known that comparable chromosome aberrations in Drosophila frequently appear as mutations by virtue of the phenotypic expression of position effects or deficiencies, the dosage-mutation rate curve should reflect this exponential trend according to the proportion of such mutations among all induced mutations. The fact that the dosage-mutation rate curve is strictly linear implies that most of the mutations measured are not the result of gross chromosome aberrations.

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STUDIES OF A TELOCENTRIC CHROMOSOME IN MAIZE WITH REFERENCE TO THE STABILITY OF ITS CENTROMERE

M. M. RHOADES*
United States Department of Agriculture, Washington, D. C.

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INTRODUCTION

EVERY chromosome possesses a differentiated region which has been designated by a variety of terms such as 'kinetochore,' 'spindle attachment region,' 'insertion region' and 'centromere.' Beginning with METZNER (1894) it has been believed that this differentiated region, which we shall call the centromere following DARLINGTON, played an essential rôle in chromosome dynamics, being involved in the congression of the chromosomes to the metaphase plate and in their poleward movements at anaphase. Conclusive proof of these probable functions, however, was obtained from the mitotic behavior of acentric fragments which arise through crossing over in heterozygous inversions or by breakage of the chromonemata, and are found to lie as passive bodies, or behave irregularly, upon the acromatic figure.

As is well known it is usually the centromere which leads the way in the anaphase movement of the chromosome towards the pole but there are a few notable exceptions. For example, in the monocentric spindles in Sciara (METZ 1938) one group of chromosomes passes away from the pole even though they show by their orientation, shape, and proximal attenuation that a force located at the centromere is being exerted upon them to move in the direction of the pole. METZ believes that the unusual behavior in these monocentric mitoses indicates the presence of two distinct forces governing chromosome movement. One of these is centered at the centromere while the other appears to be distributed through the length of the chromosome and not centered at one point. However, in the case of bipolar mitoses it is the movement controlled by the centromere which is recognizable and the second force while present and operating cannot be distinguished because of the nature of the bipolar spindle. METZ'S observations and BELAR'S (1928) studies with living spindles, in which he found evidence that a portion of the anaphase movement of the chromosomes was due to the expansion of the middle part of the spindle thus pushing the chromosomes apart, make it clear that the centromere is not the sole agent concerned in anaphase movement. That it is an essential one there is no doubt.

* The cost of the accompanying plates has been borne by the Galton & Mendel Memorial Fund.

In recent years a number of investigators, especially SCHRADER (1932, 1936, 1939) and TRANKOWSKY (1930), have become concerned with the structure of the centromere. SCHRADER describes the centromere in the amphibian Amphiuma as a compound body composed of a commissural cup enclosing a minute chromatic spherule which is directly involved in the formation of the half-spindle fiber. The chromatic spherules of SCHRADER are analogous to the kinetic bodies described by SHARP (1934). It may be questioned, however, if the structure of the centromere is identical in all organisms. In maize, where in aceto-carmine smears the centromeres of the paired chromosomes at pachytene have a homogeneous, translucent appearance, the writer has never seen convincing evidence of the presence of chromatic spherules nor were they apparent at meiotic metaphase and anaphase where they should be readily observed (see figure A, Plate 2 and figures A and B, Plate 3). SCHRADER (1939) suggests that the centromeres of Amphiuma and Zea are basically alike in structure and that the apparent dissimilarity between them at meiotic anaphase results from differences in resistance to mitotic separation. However, SCHRADER finds evidence of structural heterogeneity in the centromeres of Amphiuma chromosomes in the late meiotic prophases—a condition which has not been observed in the centric regions of Zea chromosomes, although, it must be admitted, different fixing and staining methods might disclose a more complex structure.

The centric regions of both Zea and Amphiuma exhibit staining reactions which differ from those of the rest of the chromosome. SCHRADER noted that the centrosomes and the chromatic spherules have similar staining properties and suggested a relationship between these two bodies. POLLISTER (1939) reported a striking correlation between the numbers of centrioles and centromeres in Vivapara where it appears that the centromeres may become disassociated from degenerating chromosomes and take on the properties of centrioles. Unfortunately, in maize the presence of clearly defined centrosomes and chromatic spherules has never been established. Even though the structure of the centric region may not be identical in all organisms, it is apparent that it is a well differentiated region of the chromosome which has a specialized function quite distinct from that of any chromomere.

The centromere is not concerned solely with chromosome movement. It divides the chromosome into two arms which behave in some respects as independent units since the direction of relational coiling in the two arms is at random (SAX 1936) and the genetic phenomenon of interference does not extend from one arm to the other across the centromere. It has also been established, in Drosophila particularly, that the frequency of crossing over is reduced in those regions adjacent to the centromere.

MATHER (1936) has suggested that this reduction in crossing over in the proximal regions may account for their genetical inertness and the accompanying accumulation of heterochromatin. UPCOTT (1937) believes that cell wall formation occurs under centric control. DARLINGTON (1937) believes that the centric region of the chromosome effects the structure of the spindle.

This list of suggested functions of the centromere makes no claim to completeness but it does illustrate that the centromere plays a variety of rôles in mitosis.

The terms telomitic and telocentric have been used to describe chromosomes with an apparently terminal centromere while the terms atelomitic and atelocentric indicate that the centromere is non-terminal. A number of investigators, especially S. NAWASCHIN (1916) and LEWITSKY (1931) who have stated their position clearly and unequivocally, hold that no chromosome has a true terminal centromere. It is maintained that those chromosomes which ostensibly have a single arm possess a minute second arm so small as to escape observation unless special techniques are used at critical stages. That this view may be correct is indicated by the demonstration that a number of rod-shaped chromosomes long believed to be telocentric actually have a sub-terminal and not a terminal centromere. A particularly impressive investigation has recently been made with the minute fourth chromosome of *Drosophila melanogaster* which is so small that it has a dot-like shape in oogonial cells with no indication of being other than telocentric. However, KAUFMANN (1934) from his study of the somatic prophases believed the fourth chromosome to be two-armed. Later, GRIFFEN and STONE (1939) obtained genetic and cytological evidence confirmatory of KAUFMANN'S observation. KAUFMANN also showed that the rod-shaped X chromosome of *melanogaster* has a sub-terminal centromere. The rod-shaped chromosomes of certain Orthoptera have long been held to possess terminal centromeres. DARLINGTON (1936) however states that none of the chromosomes of Chorthippus and Stauroderus possesses a terminal centromere. The joining of two rod-shaped chromosomes at the centromere to form a V-shaped element has been observed in certain Orthoptera (KING and BEAMS 1938). While this has been taken to prove that the rod-shaped chromosomes are telocentric, it is not improbable that they are similar to the X of *Drosophila melanogaster* in that they possess a minute short arm composed of genetically inert material. Unequal translocation could result in the two genetically active long arms becoming attached to a common centromere. Whether or not the two short arms were lost or retained would be of no consequence if they are composed of inert material.

There is apparently no certain case of a telocentric chromosome in the

regular chromosomal complement of any plant, and it is possible, though not as thoroughly established, that a similar condition is true among animals. The failure to find a single undoubted case of a telocentric chromosome in the regular complement of any organism suggests that a centromere so placed is either unable to function properly or is unstable (*cf.* DARLINGTON 1939). The writer (1936) described the occurrence and behavior of a supernumerary telocentric chromosome. It would appear, therefore, that the failure to find terminal centromeres in the normal chromosome complement may be due to their instability and that this instability has led to their disappearance through selection. Further study of the telocentric chromosome mentioned above has yielded data which are pertinent to the consideration of the stability of terminal centromeres.

A TELOCENTRIC CHROMOSOME IN MAIZE

Maize has a haploid set of ten chromosomes. McCLINTOCK (1933) has shown that each member of the complement can be recognized by its architecture. No member of the regular chromosome complement has a terminal centromere. The fifth longest chromosome has been associated with the *a2-bm-Pr-v2* linkage group. This chromosome has its centromere in a nearly median position. The ratio of the length of the two arms is 1.1:1.0. In certain strains of maize the longer arm often carries a prominent knob which facilitates distinguishing between the two arms. Plants trisomic for chromosome 5 differ markedly in their appearance from disomic sibs. They have thicker, broader leaves with blunter tips, a stubbier tassel, and a shorter stature than do disomes. There is no difficulty in classifying a segregating progeny into disomic and trisomic types.

In 1933 among the progeny of a plant trisomic for chromosome 5 there occurred a single plant which was intermediate in appearance between its trisomic and disomic sibs. A cytological examination of this exceptional plant disclosed that it possessed 21 chromosomes but that the extra chromosome consisted of the short arm only of chromosome 5. It had a terminal centromere. It arose through a break at or in the centromere of a normal chromosome 5. It is difficult to ascertain if the size of the centromere on the short arm of chromosome 5 is identical with that of a normal chromosome 5 because the apparent size of a centromere varies considerably in different cells. The short arm, however, does possess a readily visible centromere approaching in size that of a normal chromosome 5. While there is no proof that this terminal centromere arose from a fracturing of the parental centromere, it is of interest to note that McCLINTOCK (1932, 1938) has shown that both parts of a transversely broken centromere are capable of functioning.

Previously (1936) this chromosome was described as a 'fragment' chro-

mosome but since this term has been so widely used to denote acentric chromosomes arising either spontaneously or through irradiation the term 'telocentric' is preferred and will be used to describe it. In the 1936 paper the method was described by which the telocentric chromosome consisting of the short arm of chromosome 5 was utilized in placing the genes of the fifth linkage group in the long and short arms of the chromosome. According to the data summarized by EMERSON, BEADLE and FRASER (1935) the order with intervening crossover values of 6 of the 23 genes in this group is *a2* (6) *bm* (6) *bv* (19) *pr* (9) *ys* (32) *v2*. The available linkage data were insufficient to place accurately the remaining 17 genes. Eight of the genes in the fifth linkage group were tested against the telocentric chromosome and the *v2 ys pr v12 v3* and *bt* loci were found to lie in the long arm and *a2* and *bm* in the short arm of chromosome 5. The data presented in table 1

TABLE 1

Summary of Bm Bt Pr backcross data.

F_1 GENOTYPE	PARENTAL COMBINATIONS	REGION 1	REGION 2	REGIONS 1 AND 2	TOTAL
Bm bt pr / *bm Bt Pr*	135 462	8 3	92 268	2 2	972
		1.13%	37.04%	0.41%	
			Bm−*Bt*=1.5%		
			Bt−*Pr*=37.5%		

The inequality of the complementary classes is due to the poor germination of *bt* seed.

show that the order is *bm bt Pr* and that there is only 1.5 percent of recombination between *bm* and *bt* although they lie on opposite sides of the centromere. MCCLINTOCK (1938) from her study of ring-shaped fragments placed *bm* in the short arm close to the centromere. STADLER (1935) obtained a deficiency in the long arm of chromosome 5 which included the *v3* locus but not the neighboring loci of *bm*, *bt* or *bv*. BURNHAM (1934) reported that the order of genes going from the end of the long arm towards the centromere is *v2 ys pr bv bm*. RHOADES (1933b) from a study of a reciprocal translocation indicated that both *bm* and *bt* were close to the centromere of chromosome 5 and BURNHAM reached the same conclusion from his study of another translocation. The above cited data from various investigators give the same placement of loci arrived at by the use of the telocentric chromosome. Utilizing the cytogenetic data it is possible to place both *bt* and *v3* in the linkage map which becomes:

0	6	8	10	12	31	40	72
a2	*bm*	*bt*	*v3*	*bv*	*pr*	*ys*	*v2*

CYTOLOGICAL STUDIES

Synapsis of the telocentric chromosome with the two normal chromosomes 5 was studied in plants hyperploid for the telocentric chromosome. A number of clear pachytene figures was obtained in which the synaptic relationships could be determined (figure 1). At any given region pairing between the three homologous short arms was always in twos, with the

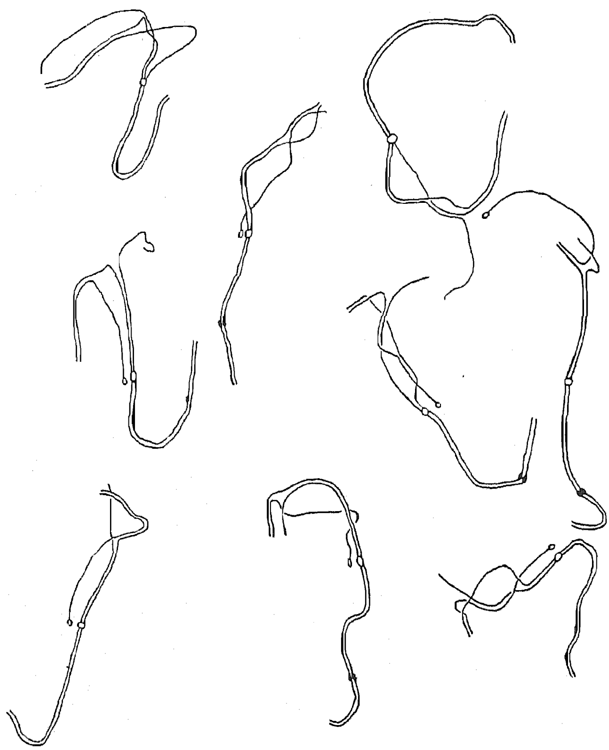

FIGURE 1.—Camera lucida drawings at pachytene showing synapsis of telocentric chromosome with the two normal chromosomes 5. The centromeres are represented by clear ovals or circles and the prominent knob in the long arm, when present, by dark ellipses.

third arm unpaired. Exchanges of pairing mates among the three arms were not frequent; in the majority of pachytene figures there was a single exchange of partners and the greatest number observed was three. It should be clearly understood that the exchange of pairing mates referred to is between the three homologous arms and should not be taken to indicate the existence of chiasmata. It is, of course, true that the observed exchange of partners must occur before chiasmata can be formed between the different chromosomes, but there is no reason to believe that a chiasma is formed in every paired segment lying between the points where exchanges of partners occur, especially if the paired region is short. In fact,

if the partial chiasmatype theory of crossing over is correct, which seems likely, it is not proper to define a chiasma as consisting of an exchange of the pairing elements (that is, chromatids), since sister chromatids are paired on both sides of a chiasma; all that has occurred is a breakage and reunion of ends between two non-sister chromatids. The term exchange of partners is used correctly to describe the exchange of partners among homologous chromosomes in polyploids or in the case of a reciprocal translocation where structural dissimilarity causes a change of mates.

It is evident from figure 1 that pairing between the telocentric chromosome and a normal chromosome 5 does not necessarily commence at the centromere, as the terminal centromere often lies to one side of the two paired centromeres of the normal chromosomes 5; and in some cases was observed 'stuck' to the centromeres of other pairs of chromosomes without preventing synapsis in distally placed regions.

Plate 2, figure B is a photomicrograph of an unpaired telocentric chromosome at pachytene. The equational division or split of this chromosome into chromatids is evident at the distal end. The terminal centromere, which unfortunately cannot be clearly seen in the photograph, appears to be divided or possibly is beginning to divide inasmuch as its distal end is cleft or heart-shaped. As the writer stated in 1936, this apparent division of the terminal centromere at mid-prophase may or may not be representative of the behavior of paired centromeres interstitially located. In a number of organisms the genetic and cytological evidence is convincing that the first meiotic anaphase is reductional for the centric regions of bivalent chromosomes; irrespective of the physical state of division of the centric region of two sister chromatids it acts as a single functional unit. As figure A, Plate 3 suggests, and indeed as has been reported by both SCHRADER and DARLINGTON, each chromatid of the metaphase tetrad may give rise to its own half-spindle component. But SCHRADER points out that it is the chromatic spherules in Amphiuma which give rise to the half-spindle components, and while each chromatid has its own chromatic spherule the commissural cup in which they both lie has not divided so the disjunction of the sister chromatids at the centric region is necessarily reductional. Figure B, Plate 3 also indicates that the bulk of the centric region has not divided even though it clearly shows that each chromatid will form what SCHRADER terms its half-spindle component.

The frequency of trivalent association at metaphase I between the telocentric and the two normal chromosomes 5 was determined in microsporocytes. Metaphase counts were made in five plants and the mean frequency of trivalent association found to be 59 percent. The frequency of trivalents ranged from 50 to 70 percent in different plants. Whether or not this difference is genetic or caused by environmental factors cannot be stated,

but that it is genetic is suggested by the fact that different anthers of one plant gave consistently higher values than did anthers of another. Genetic data to be reported in a later section on the difference between the frequencies of plants hyperploid for the telocentric chromosome in two strains segregating for disomic and hyperploid individuals also argues for a genetic basis.

Figure E, Plate 2 is a photomicrograph of a trivalent group at metaphase I which is typical of the majority of cases where a trivalent occurs. The telocentric chromosome is oriented on the spindle in such a way that it will pass to a pole with one of the normal chromosomes 5, which will disjoin from each other. This non-random orientation of the trivalent, which leads to non-random distribution, is a natural and logical consequence of the equilibrium position attained by the interaction of the three centromeres, which tend to repel each other, and of the chiasmata by which the association of paired chromosomes is maintained through metaphase. In most cells one chiasma, at least, is formed between the two long arms of the two normal chromosomes and one between their two short arms. If in addition a chiasma is formed between the telocentric chromosome and one or other of the two short arms of the normal chromosomes 5, this latter chiasma comes to occupy a terminal position because of the generalized repulsion existing at this time between chromosomes as bodies, in addition to the localized centromere repulsion (*cf.* DARLINGTON 1937). Since both arms of the two normal chromosomes remain associated by chiasmata they will tend to lie symmetrically upon the spindle with their centric regions oriented towards opposite poles. The telocentric chromosome which is associated distally with the normal chromosomes by a triple terminal chiasma will lie more or less in the longitudinal axis of the spindle (that is, at right angles to the equatorial plate) with its terminal centromere directed poleward. The end result is that the centromeres of the two normal chromosomes are oriented against one another while that of the telocentric chromosome is not subject to such regulation. The type of disjunction is not determined by the centromeres themselves. Their orientation on the spindle is a function of the metaphase configuration produced by prophase pairing and chiasma formation. Such metaphase configurations as were commonly observed would be expected to produce anaphase disjunctions in which the two normal chromosomes 5 disjoin from each other with the telocentric chromosome accompanying one or other of the normals. These configurations rarely should give an anaphase distribution in which the two normals pass to the same pole while the telocentric chromosome goes to the opposite one. The genetic data in tables 2–5 amply confirm this expectation.

When the telocentric chromosome is a member of a trivalent group it passes poleward at the same time at which the bivalents are disjoining. It is recognizable during anaphase I because, possessing a terminal centromere, it has a V-shaped appearance resulting from the attachment of the two chromatids at the undivided centromere while their distal ends are some distance apart. The other dyads in anaphase I have a double V- or double J-shaped appearance, depending upon the relative lengths of their two arms, with the apices of the V's or J's attached to a common centromere.

In prophase II the two monads of the telocentric chromosome, which will separate equationally in the subsequent anaphase, form a single rod-shaped chromosome as they are conjoined by the still undivided centromere. The repulsion between the two chromatids is so pronounced at this stage that they tend to lie in a straight line. The centromere does not appear in its usual position but is forced to one side and the two chromatids appear fused at their proximal ends. This specious appearance is even more clearly illustrated in the case of the other dyads, especially those where the two arms differ greatly in length. Here the two short arms appear joined into a single element while the two long arms also appear united into a single body, the two elements being separated by the centromeric region (figure G, Plate 3). When the equational separation of the two chromatids of the telocentric dyad occurs in anaphase II, the monads (chromatids) appear as rod-shaped bodies with the terminal centromere leading the way to the pole. The other monads appear as single V's or J's, depending upon the location of the centromere (figure J, Plate 2).

The unpaired telocentric chromosome usually lay on the spindle at metaphase I but in some cells failed to move onto the plate (figure G, Plate 2). Its behavior at anaphase was variable. After the bivalents had completed their anaphase movements, its two halves often would separate equationally and the daughter univalents would begin their migrations to opposite poles. That they were able occasionally at least to complete their journey to the poles in time to be included in the interphase nuclei was made evident by the observation of daughter univalents in prophase II. The equational separation of the univalent occurs so much later than the disjunction of the bivalents that the writer previously (1936) believed they failed to divide equationally in the first meiotic division, but this was an erroneous conclusion drawn from the study of too early anaphases. While the univalent usually divided equationally in anaphase I, it did not always do so because it was not uncommon to find the chromosomes at the two poles in interphase with the univalent lying to one side in the cytoplasm. Presumably it was those univalents that congressed which

later divided equationally at anaphase while those that failed to congress become laggards. When they lie near a pole, lagging univalents may in some cases be drawn fortuitously into the telophase nucleus.

When a daughter univalent succeeded in reaching the pole in anaphase I, it lagged in the following anaphase because it had precociously undergone the equational separation which normally occurs in the second division (figures H, I, Plate 2). No detailed counts were made but it seemed that the number of lagging chromosomes at anaphase II was less than twice the number of univalents which split equationally in the first division. UPCOTT (1937) noticed a similar phenomenon in Tulipa and suggested that some of the daughter univalents were carried to the poles in anaphase II along with the dividing chromosomes. Another possibility is that the daughter univalents which do not reach the poles in anaphase I fail to congress at metaphase II and, lying off of the spindle, would not appear to be lagging. This point is worthy of more study, but the cytological observations indicate that the telocentric chromosome was almost invariably lost through lagging at the first or second meiotic divisions when it was unpaired at metaphase I. The genetic data on the frequency of hyperploid individuals in the progeny of a hyperploid plant is the best evidence that the unpaired telocentric chromosome usually suffered elimination. Conversely, the telocentric chromosome underwent normal meiotic behavior when it was a member of a trivalent group. In brief, the cytological observations show that (1) the usual orientation of the trivalent group on the metaphase plate was of such a nature as to lead to a non-random distribution in anaphase. The gametes should consist mainly of two types, namely those that are haploid and those hyperploid for the telocentric chromosome, with relatively few gametes having two normal chromosomes 5 or the telocentric chromosome only. (2) The observed frequency of trivalents at metaphase permits the prediction of the relative numbers and types of offspring expected in the progeny of a plant hyperploid for the telocentric chromosome, since the unpaired telocentric is usually eliminated when it is an univalent.

GENETIC STUDIES WITH THE TELOCENTRIC CHROMOSOME

The *a2* and *bm* loci were reported by the writer (1936) to lie in the short arm of chromosome 5. A strain was obtained hyperploid for the telocentric chromosome carrying the recessive *bm* allele in each of the normal chromosomes 5 and the dominant allele in the telocentric chromosome. These hyperploid plants were crossed reciprocally with diploid *bm* individuals and the progeny classified for the *bm* character and the different chromosomal types. Table 2 summarizes the results obtained using the hyperploid individuals both as the male and female parent in backcrosses. The data

show that 98.91 percent of the progeny obtained when hyperploid plants were used as the male are diploids homozygous for the recessive allele *bm*. These arose from the functioning of a haploid pollen grain carrying *bm* in a normal chromosome 5. Eighteen of the 7,245 plants or 0.25 percent were diploids not exhibiting the *bm* character and therefore carried the dominant allele in the chromosome contributed by the hyperploid parent. This type of chromosome arose from a crossover between the telocentric chromosome and a normal chromosome in the *bm*-centromere interval so that the dominant allele was transferred to the normal chromosome. Thirty-seven or 0.51 percent of the offspring were hyperploid for the telocentric chromosome and were identical in constitution with the male parent. These individuals arose through the functioning of a pollen grain carrying the telocentric chromosome with the dominant allele and a normal chromosome 5 with a recessive allele. As the cytological observations show that something over thirty percent of the grains should be hyperploid for the telocentric chromosome, the genetic data show that the hyperploid grains do not successfully compete with haploid pollen and that it is only an occasional hyperploid grain which effects fertilization. Hyperploid pollen grains are well filled with starch and cannot be distinguished in appearance from haploid grains. The ineffectiveness of hyperploid grains in accomplishing fertilization in competition with haploid pollen is probably due to a slower rate of pollen tube growth.

Two *bm* individuals were primary trisomes of chromosome 5. These plants arose from a gamete with two normal chromosomes 5. If they were contributed by the male it follows that two events must have occurred. First, the disjunction in anaphase I must have been such that the two normal chromosomes 5 went to the same pole. That such disjunction occurs is shown by the data in the same table where 1.63 percent of the eggs received two normal chromosomes 5. Second, such a hyperploid pollen grain with two normal chromosomes 5 must have functioned in competition with haploid pollen. That such grains occasionally do compete successfully was shown in the experiment in which related primary trisomes of chromosome 5 were used as the male parent, five plants in the total of 1,212 offspring being primary trisomes. The product of the frequency of the two events gives a probability of less than one such individual expected where two were observed. There is also the possibility that an egg with two chromosomes 5 arose in the diploid female parent through non-disjunction. Such spontaneous occurrences of primary trisomes have been observed but they are so rarely found that nothing is known of their frequency. It seems not unreasonable to suppose that the two primary trisomes arose from a male gamete in the manner suggested.

The foregoing classes with their observed frequencies can be readily ac-

counted for. In addition to them, however, there is a class which is entirely unexpected as it includes a type of chromosome absent in either parent. This class consists of the 22 secondary trisomes which possess a supernumerary chromosome consisting of two short arms of chromosome 5 attached to a single, median centromere. Twenty-one of the secondary trisomes were *Bm* and one was *bm*. This suggested that the telocentric chromosome was involved in the formation of the secondary or iso-chromosome since it carried the *Bm* allele. The exceptional *bm* secondary trisome

FIGURE 3.—The seedling to the left is a secondary trisome in which the secondary chromosome is composed of two short arms of chromosome 5 attached to a median centromere. The secondary chromosome arose through misdivision of the centromere of a telocentric chromosome consisting of the short arm of chromosome 5. The seedling to the right is a sibling of the secondary trisome and is a diploid. The differences in growth and texture of the leaves are quite pronounced between the secondary trisome and its disomic sib.

could be accounted for by a crossover between the telocentric chromosome and a normal chromosome 5. It also is possible that it arose from a normal chromosome 5 by transverse division of the centromere. Data presented in other tables leave no doubt that the telocentric chromosome is involved in the formation of the new type of chromosome. The secondary trisomes were strikingly different in appearance from any of the other chromosomal types. Their dwarf-like habit and thick leaves of leathery texture made them easily recognizable as seedlings (see figure 3) while the other chromosomal types could not be accurately classified until a much later stage.

Hyperploid plants used as female parents in backcrosses with diploid *bm* individuals yielded progenies distinctly different in some respects from those obtained when the hyperploid plants were used as the male parent. As table 2 shows, there were 10 diploid *Bm bm* plants out of a total of 5,523, or a percentage of 0.18. This value agrees very well with the percentage of 0.25 for the same class when the hyperploids were used as the male. These plants originate as before from a crossover between the telocentric chromosome and a normal chromosome 5 in the *bm*-centromere region. There were 3,738 (67.68 percent) diploid *bm* individuals. This class comes from haploid gametes with non-crossover chromosomes. The frequency of this class is much lower than in the reciprocal backcrosses as there were 1,671 or 30.26 percent hyperploid plants while only 0.51 percent of the offspring were hyperploid *Bm* plants when hyperploid individuals were used as the male parent. The difference between the two kinds of backcrosses in the frequency of the hyperploid *Bm* is due to the fact that there is little or no competition between megaspores. If the basal megaspore of the quartet happens to receive the telocentric chromosome in addition to a normal chromosome 5, it develops without competition into a functional embryo sac; but a hyperploid pollen grain never enjoys such a positional advantage and must compete against haploid spores. The data obtained when hyperploid plants were used as female parents also differ from the reciprocal cross in that five hyperploid individuals with the recessive *bm* allele in all three chromosomes were found in the former while none were observed in the latter data. These five hyperploid *bm* plants arose from a crossover which transferred *bm* from a normal chromosome to the telocentric chromosome which later passed to the same pole as a *bm*-bearing normal chromosome 5. Similar gametes arising in microsporogenesis would fail to survive because of their relative inability to function in competition with haploid spores. Likewise 1.63 percent of the plants were primary trisomes homozygous for *bm* in progenies obtained when hyperploid plants were used as female parents while only 0.03 percent were found in similar progenies when the hyperploid plants were used as the male parents. There is no reason to believe that the actual frequency of such gametes is greatly different in the two sexes; it is simply a matter of the presence or absence of competition from haploid spores. The number of *bm* primary trisomes obtained when hyperploids were used as female parents is a measure of the frequency with which the two normal chromosomes 5 go to one pole while the telocentric chromosome passes to the other. Likewise the number of individuals hyperploid for the telocentric chromosome is a measure of the frequency with which one of the normal chromosomes passes to the same pole as the telocentric chromosome. The data show that there were 90 of the former to 1,671 of the latter type

of disjunction. This is precisely the type of behavior predicted from the cytological study of pairing and orientation on the spindle at metaphase I. As in the crosses where the hyperploid plants were used as the male parent, there occurred the unexpected class of secondary trisomes. Nine secondary trisomes were found; all of them were *Bm* which indicates that the telocentric chromosome was involved in the formation of the secondary chromosome.

The *bm* locus lies in the short arm of chromosome 5 close to the centromere. Distal to *bm* lies the *a2* allele some 10–12 crossover units removed. Data from plants carrying the dominant allele of the *a2* locus in the telocentric chromosome and recessive alleles in the two normal chromosomes 5 should parallel those reported for *bm* except that the greater crossover distance of *a2* from the centromere should alter the relative frequency of certain classes of offspring. These data are given in table 3. They agree very well with those for *bm*. The percentage of diploids with the dominant allele is 1.74 and 0.82 when hyperploids were used as the male and female parents, respectively. The *A2*-bearing normal chromosome arises through crossing over in the *A2*-centromere interval. The higher frequency of diploid *A2 a2* offspring obtained when hyperploids were used as the male parent in backcrosses suggests a higher crossover value in male than in female flowers. This is in agreement with unpublished data of the writer's which show for chromosome 5 significantly higher crossover values in male flowers as compared with the female. In both types of backcrosses the percentage of *A2 a2* individuals is several times greater than the percentage of *Bm bm* plants. This follows from the fact that *a2* is further removed than *bm* from the centromere.

In the data obtained when hyperploids were used as male parents, the percentage of hyperploid plants with the telocentric chromosome carrying *A2* is 0.42. In addition there were 2 (0.04 percent) individuals hyperploid for a telocentric chromosome with *a2*. These arose through crossing over followed by the functioning of a hyperploid grain and would consequently be found rarely. The total percentage of individuals hyperploid for the telocentric chromosome is 0.46 which is similar to the percentage of 0.51 in table 2 for the *bm* data. One primary trisome homozygous for *a2* was found. It could have arisen spontaneously in the diploid female parent or from the functioning of a pollen grain with two normal chromosomes 5 each with *a2*. As in the case of those hyperploid plants used in the *bm* experiments these also throw the unexpected class of secondary trisomes. There were 26 secondary trisomes in a total population of 5,450 or a percentage of 0.48 when hyperploid individuals were used as male parents. All 26 secondaries were *A2* in appearance which supports the conclusion

drawn from the *bm* data that the telocentric chromosome is involved in the formation of the secondary chromosome.

In the offspring of hyperploids used as the female parent 1,411 in a total of 5,605 individuals, a percentage of 25.17, were hyperploid for the telocentric chromosome. Thirteen hundred and eighty-six of them were phenotypically *A2* and 25 had *a2* in the telocentric as well as the normal chromosomes 5. Since the *A2* locus affects aleurone color an attempt was made to determine the genotypic constitution of all 1,386 plants hyperploid for the telocentric chromosome and carrying an *A2* allele by pollinating them with *a2* pollen. Successful pollinations were made on 1,159 plants. In all but one plant the aleurone ratios indicated the presence of a single *A2* allele borne by the telocentric chromosome. The single exception had *A2* in both the telocentric and a normal chromosome 5. This exceptional plant is a consequence of a crossover in the *A2*-centromere region between the telocentric and a normal chromosome, following which the two chromosomes involved in the crossing over passed to the same pole at anaphase I. One of the four possible combinations formed at the end of the second division would have an *A2* allele in both the telocentric and the normal chromosome 5. The 25 *a2* plants hyperploid for the telocentric chromosome also arose from crossing over in the *A2*-centromere region. If, following a crossover between the telocentric and one of the two normal chromosomes in this region, the assortment of the telocentric chromosome is at random with respect to the two chromosomes 5, there will result 3 $\frac{a}{a}$ to 1 $\frac{A}{A}$ combinations at the end of the second division. If the two crossover chromosomes always disjoin to opposite poles the expected ratio is 2:0 while a ratio of 1:1 is expected if they always pass to the same pole. The observed ratio of 25:1 indicates that the two crossover chromosomes usually pass to different poles. This correlation between crossing over and disjunction is similar to that found in triploid Drosophila. It should be mentioned that of the 1,385 individuals listed in table 3 as having their single *A2* allele in the telocentric chromosome only 1,158 were actually proven by genetic tests to be so constituted. However, since only one exceptional individual was found in the total of 1,159 tested, little error is introduced by this classification.

The percentage of *a2* plants hyperploid for the telocentric chromosome is 0.45 and the percentage of *bm* plants of similar chromosomal constitution, from table 2, is 0.09. This difference can be ascribed to the relative positions of the two loci with respect to the centromere. There were 48 primary trisomes homozygous for *a2*. This percentage of 0.86 is approximately

half the percentage of *bm* primary trisomes from a similar type of cross. The total frequency of individuals hyperploid for the telocentric chromosome in the *a2* data is 25.17, which is lower than the percentage of 30.35 found in the *bm* data. The reduced percentages of both primary trisomes and plants hyperploid for the telocentric chromosome in the *a2* data compared to the *bm* data suggest a lower frequency of pairing of the telocentric chromosome with the normal chromosomes 5 in the *a2* strain than in the *bm* strain. It is likely that this difference is genetically conditioned but nothing is known of its basis.

There were eight secondary trisomes in the progenies obtained using hyperploids as the female parent. All eight possessed the *A2* allele which again indicates that the telocentric chromosome was involved in the genesis of the secondary chromosome.

Data obtained from hyperploid plants possessing the dominant allele in the telocentric chromosome and the recessive allele in each of the normal chromosomes are the most illuminating since the dominant allele serves as a marker for the telocentric chromosome. However, in addition to these data a number of progenies were obtained from hyperploid plants which had a dominant allele in one of the two normal as well as in the telocentric chromosome. Data from a single combination of this type are presented in table 4. They will not be discussed in detail as they confirm in all respects the conclusions reached from the data in tables 2 and 3. The chief point of interest in these data is that secondary trisomes arise with a low but consistent frequency whenever the telocentric chromosome is present.

The data presented on the inheritance and behavior of the telocentric chromosome have been derived from individuals in which a single locus, either *a2* or *bm*, was followed in the telocentric chromosome. Data were obtained, in addition, from hyperploid plants in which both the *a2* and *bm* loci were marked by mutant alleles and the two long arms of the normal chromosomes were carrying the *Pr* and *pr* alleles.

The dominant allele at the *a2* locus produces aleurone color in the presence of *A*, *C* and *R* while *a2* results in colorless aleurone. In the hyperploid plants used these three complementary genes were homozygous dominant and only the *a2* locus was heterozygous, so a classification for *A2* and *a2* could be made before planting. The *Pr* and *pr* gene pair determines whether the color is to be purple or red, *Pr* conditioning purple and *pr* red color. In *a2* seeds it is impossible to classify for the *Pr pr* pair. The *a2* locus is also concerned in plant color so a check on the aleurone classification into colored and colorless was possible.

The constitution of the hyperploid plants for the three loci was *A2 Bm* /*A2 bm Pr*/*a2 bm pr*; the composition of the telocentric chromosome being listed first. These hyperploid plants were pollinated by triple recessive

pollen and the resultant seed divided into purple, red, and colorless classes. The ensuing progenies from the three classes of seed were classified for the *bm* character and for the various chromosomal types. These data are given in table 5. As a consequence of *Pr* lying in the same chromosome with *A2* while *pr* is in the *a2* bearing chromosome, the purple and red seed produced different percentages of the various classes. Among the progeny from *Pr* seed there were 471 diploid *bm* plants and only 199 from *pr* seed. The *A2 bm Pr* plants arose from non-crossover chromosomes (crossovers between the *A2 bm Pr* chromosome and the telocentric chromosome in the *A2 bm* region could not be detected) while the *A2 bm pr* individuals arose from crossovers between *pr* and *A2*. Equal numbers of plants hyperploid for the fragment should be found in the *Pr* and *pr* classes if the telocentric chromosome shows no preference with which normal chromosome 5 it disjoins in anaphase I. There were 281 and 350 hyperploid plants in the *Pr* and *pr* classes, respectively, which indicates no pronounced preferential assortment although the deviation of 35 from equality is somewhat suggestive. Another striking difference between the *Pr* and *pr* progenies is that there were 50 primary trisomes homozygous for *bm* among the *Pr* individuals while only four were found in the *pr* class. Those in the *Pr* class can be simply accounted for by non-disjunction of the two normal chromosomes. The four primaries homozygous for both *pr* and *bm* likewise arose from non-disjunction of the two normal chromosomes, but the fact that they came from gametes with two chromosomes 5 each with the recessive *pr* gene which was present in but one of the parental chromosomes indicates that a crossover in the *pr*-centromere interval took place between the chromatids of the two normal chromosomes followed by their non-disjunction in the first meiotic division. Since anaphase II is equational for the centromere, one-fourth of the combinations should carry two *pr* chromosomes while three-fourths should be *Pr Pr* and *Pr pr* in the ratio of 1:2 respectively. Approximately 12 of the 50 primary trisomes in the *Pr* class arose, therefore, in such a manner. There were two secondary trisomes present in both the *Pr* and *pr* classes. Of the 675 plants from *a2* seed all but two were diploids homozygous for *bm*. The two exceptions were hyperploid plants with a telocentric chromosome carrying *Bm*. These arose from a crossover in the *A2-Bm* region between the telocentric chromosome and the *a2-bm-pr* chromosome followed by their non-disjunction in anaphase I. One of the four possible combinations formed at anaphase 2 would possess a telocentric chromosome with the *a2 Bm* alleles and a normal chromosome of *a2 bm* constitution.

The genetic data presented in tables 2 to 5 inclusive are of interest in two respects. First, since it was possible to recognize all of the various chromosomal types arising as products of the meiotic process, extensive

genetic data were obtained which afforded a check on the observed cytological behavior of the telocentric and the two normal chromosomes in meiosis. Second, and of more interest since it is pertinent to the question of the stability of the terminal centromere, the data show that the telocentric chromosome was regularly involved in the genesis of a new chromosome equivalent to two short arms of chromosome 5 with a single, median centromere. Since adequate data have been presented on the frequency with which this new chromosome type arises, we will next consider the manner in which it originates.

ORIGIN OF SECONDARY CHROMOSOME

When plants hyperploid for the telocentric chromosome were used as the male parent a grand total of 19,242 offspring was obtained of which 86, or 0.45 percent, were secondary trisomes. Of a total of 17,175 offspring obtained when the hyperploid plants were used as the female parent there were 27, or 0.16 percent, secondary trisomes. This comparison is unfair since approximately 30 percent of the offspring in the latter crosses consist of hyperploids similar to the female parent, while less than one-half of one percent of such individuals were found when the hyperploids were used as the male parent. If the frequency of secondaries is calculated from the data obtained with the hyperploids as the female parent (omitting the hyperploid class from the total) the percentage of secondaries is 0.22. This value is about half that obtained when the hyperploid was the male parent. The relative frequencies of secondaries in the direct and reciprocal backcross data are in striking contrast to those of the other hyperploid types, namely the classes hyperploid for a telocentric chromosome or a whole chromosome 5. Although approximately 30 percent of the pollen grains possessed a supernumerary telocentric chromosome, only 0.46 percent of the offspring were hyperploid for this chromosome. It is certain that the hyperploid grains are at a great disadvantage against haploid pollen and only rarely succeed in functioning. Data have also been presented which show that grains hyperploid for a normal chromosome 5 are rarely functional in competition with haploid grains. It is likewise certain that there is little or no competition between euploid and aneuploid megaspores since the frequencies with which the different chromosomal types appear in the progenies obtained when the hyperploid is the female parent are reasonably close to those expected on the basis of pairing and disjunction at the first meiotic division in the microsporocytes. We are then faced with an anomalous situation in the frequency with which secondary trisomes appear when plants hyperploid for the telocentric chromosome are used as the pollen parent. There is no reason to believe that the frequency with which the secondary chromosome arises is enough

higher in the male flowers to account for the relatively high number of secondaries transmitted through the pollen, especially in view of the fact that pollen hyperploid for either the telocentric chromosome or chromosome 5 rarely functions even when present in large numbers. The possibility that two extra short arms of chromosome 5 have no detrimental effect on the pollen, while a single extra short arm is highly deleterious, and consequently that grains hyperploid for this secondary chromosome are as capable of functioning as haploid grains seems most unlikely. It is rendered untenable by the following experiment.

The secondary trisomes proved to be highly sterile in both the male and female flowers. Although the anthers have few aborted grains they are rarely extruded from the glumes and consequently shed no pollen. If, however, the mature anthers are removed and the pollen manually extracted, small quantities of viable grains can be obtained. When this pollen was applied to diploid silks a number of seeds were obtained. A total of 623 plants were grown from such seed and all proved to be diploids. It follows that those pollen grains hyperploid for the secondary chromosome were not able to function against haploid pollen. That they were present was proved by a study of the chromosomal complement of microspores at the first microspore division. Their frequency was not ascertained, however, because it was not always possible to differentiate between a supernumerary secondary chromosome and an extra normal chromosome 5. However, approximately 10 percent of the female progeny of a secondary trisome consist of secondary trisomes, so it is not unreasonable to assume that a like percentage, at least, of the pollen grains were hyperploid for the secondary chromosome. The failure to find a single secondary trisome in the offspring of a secondary used as the male parent makes it reasonably certain that the secondary chromosome so upsets the normal balance that the hyperploid grains are unable to successfully compete with haploid grains.

Before suggesting two possible mechanisms whereby the secondary chromosome may be transmitted through the pollen, it may be pertinent to consider the development of the male gametophyte which has been studied by a number of investigators. Essentially the story is as follows: The nucleus of the microspore divides to form a generative and a vegetative or tube nucleus. The generative nucleus divides again to form the two sperm cells. The mature male gametophyte or pollen grain at the time of anthesis contains three haploid nuclei—the vegetative nucleus, which is in a metabolic condition, and the two sperm nuclei. When the pollen grain germinates a pollen tube is extruded through the germ pore, enters the silk and grows down the silk towards the ovule. The vegetative nucleus assumes a position near the growing tip of the pollen tube and it is be-

lieved that the growth of the tube is under its control. It has been assumed that the two sperm are passive bodies playing no effective role in the activities of the pollen tube, merely being transported down the silk to the embryo sac.

The transmission of the secondary chromosome through the pollen may be readily accounted for if it is assumed that in a microspore with a telocentric chromosome the sequence of events is as follows: In the first microspore mitosis the telocentric chromosome splits equationally into two chromatids. Normally at anaphase each of these two chromatids possesses its own centromere and they pass to opposite poles. Rarely, however, the terminal centromere of the telocentric chromosome either fails to divide or divides transversely so that the two chromatids find themselves attached at their proximal ends to a common centromere. At anaphase this newly constituted chromosome with a median centromere and two identical arms passes to either the vegetative or generative pole. In the event that it moves to the generative pole, the end of the first microspore division finds a haploid vegetative nucleus and a generative nucleus hyperploid for the secondary chromosome. The two sperm formed by the division of the generative nucleus will each carry the secondary chromosome. A pollen grain of this constitution presumably would not be under any handicap during its stylar journey because it possesses a haploid vegetative nucleus. It carries, however, a sperm which will give rise to a secondary trisome of it fertilizes a haploid egg.

The postulated mis-division of the centromere of the telocentric chromosome has never been observed by the writer at anaphase of the first microspore division. DARLINGTON (1940), however, states that he observed the formation of iso-chromosomes in microspores of Fritillaria resulting from the delayed division of newly arisen telocentric chromosomes. An attempt was made to observe the phenomenon cytologically but the low frequency of its occurrence (about 9 in a 1,000 judging from the number of secondaries in the offspring) and the difficulty of identifying individual chromosomes at the microspore anaphase proved to be insuperable difficulties. While it was not possible to observe the genesis of the secondary chromosomes in the manner postulated the evidence at hand suggests that this mechanism or a similar one gives rise to the secondary chromosome.

In addition to the above hypothesis there is another possible way in which the telocentric chromosome might give rise to the secondary chromosome. KOLLER (1938), UPCOTT (1937) and especially DARLINGTON (1939) have shown that the centromere of an univalent chromosome sometimes divides transversely at meiosis in such a way that the two short arms are joined together and the two long arms are also attached to one piece of centromere. That is, the misdivision of the centromere gives rise to two

isochromosomes. In the case of the telocentric chromosome it has been observed that it is often unpaired by meiosis and that it sometimes fails to split equationally in anaphase I. It is possible, though it has not been cytologically demonstrated, and indeed it would be difficult to do so with certainty with a telocentric chromsome, that the centromere of an occasional telocentric chromosome divides transversely in either the first or second meiotic divisions to form an isochromosome with two identical arms. This newly formed isochromosome fails to reach either pole and forms a micronucleus. It must be further assumed that it persists until the microspore division where through its fortuitous position in the cell it is occasionally incorporated into the telophase group at the generative pole. The end result here is the same as in the first hypothesis, namely that the vegetative nucleus is haploid while the generative nucleus is hyperploid for the iso- or secondary chromosome. This hypothesis has the advantage that the misdivision of the centromere is postulated to occur in the meiotic divisions, where univalents of other plants have been observed to misdivide, and not in the somatic division of the microspore. While it has been necessary to invent the two hypotheses primarily to account for the transmission of the secondary chromosome through the pollen, it is highly probable that whatever mechanism is responsible for the origin of the secondary chromosome in the male flowers is also responsible for its origin in the female flowers.

The secondary trisomes originating from plants hyperploid for the telocentric chromosome have been studied cytologically. Figures C, D, E and F, Plate 3 and figures A, B and C, Plate 1 show that the secondary chromosome is composed of two short arms of chromosome 5 with a median centromere. A study of synapsis reveals that the order of loci in the secondary chromosome is *a b c d e* centromere *e d c b a* which is the order expected from the postulated mechanisms. When the secondary chromosome is a univalent but forms a chiasma between its two homologous arms a ring of one is found at diakinesis (figure C, Plate 3). The associations of the secondary and the two chromosomes 5 at pachytene shown in figures A, B and C, Plate 1 are explicable only if the 'secondary' chromosome consists of duplicate arms. The cytological behavior of these secondary trisomes is similar to that of the secondary for the short arm of chromosome 5 which arose spontaneously (RHOADES 1933a) in a stock disomic for chromosome 5. The genetic data disclose that the telocentric chromosome is involved in the formation of the secondary chromosome since with one exception (which can be accounted for by a crossover) the same alleles are present in the two chromosomes. (No secondary trisomes were found in the thousands of offspring from disomic sister plants.) These data also indicate that pollen hyperploid for one or two short arms of chromosome

5 rarely functions, yet the frequency of secondaries transmitted through the pollen is certainly no less than through the eggs. All of the above enumerated facts point to the correctness of the hypothesis that the secondary chromosome arises through mis-division of the centromere of the telocentric chromosome and as a consequence of this mis-division the generative and vegetative nuclei of the male gametophyte differ in their constitution.

Irrespective of the precise manner in which the secondary chromosome arises, it is a reasonable inference that its formation results from the instability of the terminal centromere of the telocentric chromosome. Whether its misbehavior consists of failure to divide or of a transverse instead of a longitudinal division cannot be stated with certainty but the observations of KOLLER (1938), UPCOTT (1937) and DARLINGTON (1939) on the transverse centromere division of univalent chromosomes at meiosis make the latter probability more likely. Evidence has been presented which suggests that in the formation of the secondary chromosome the mis-division of the terminal centromere occurs during or immediately following meiosis. An experiment was undertaken to determine if the telocentric chromosome was unstable in sporophytic mitoses. When plants hyperploid for the telocentric chromosome with the *Bm* allele, the two normal chromosomes 5 carrying *bm*, are used as female parents approximately 30 percent of the offspring are hyperploid for the telocentric chromosome. These individuals are *Bm* phenotypically because the telocentric chromosome bears the dominant allele. If, however, the telocentric chromosome or that part carrying the *Bm* locus is lost during the development of the sporophyte the recessive brown mid-rib character is expressed in the deficient portions of the plant. Three hundred hyperploid individuals were closely examined for the presence of *bm* sectors, and 22 or 7.3 percent were found possessing them. In these 22 plants the *Bm* allele present in the telocentric chromosome had been eliminated during development of the sporophyte. In several of the plants the deficient sectors extended into the tassel and a cytological examination was made of microsporocytes lacking the *Bm* allele. In one case the telocentric chromosome had been completely eliminated while in four other plants the telocentric chromosome had become diminished in size. In two of these instances there was a small fragment with a subterminal centromere; a second plant had a chromosome with a terminal centromere but only half the length of the parental telocentric chromosome; the third had an extremely small chromosome fragment consisting of nothing more than a terminal centromere with two or three chromomeres.

In the few cases studied cytologically no indication was found that the *bm* variegation was due to a reciprocal translocation occurring in a somatic

cell and resulting in somatic segregation such as JONES (1938) reports for the endosperm of maize.

A similar experiment was conducted in which the telocentric chromosome was marked with the dominant *A2* allele while the normal chromosomes 5 carried the recessive allele. The *B* and *Pl* alleles were also present in this stock so the *A2 B Pl* plants had a purple plant color. If *A2* was lost during the development of the sporophyte the tissue deficient for this allele would be brown instead of purple. Five hundred and one purple hyperploid plants were examined at maturity for brown sectors and 31 or 6.2 percent possessed them. The *a2* sectors must have arisen through the loss of *A2* in telocentric chromosome. The size of both *bm* and *a2* sectors varied from small to large. No cytological study has as yet been made of the *A2* losses. It appears, however, from the study of certain of the *Bm* losses that an unchanged telocentric chromosome was present in the early embryo and that some alteration occurred during development because the non-deficient cells possessed a complete telocentric chromosome while the deficient cells had a reduced or missing telocentric chromosome. If elimination of the telocentric chromosome in somatic tissue sometimes occurs through the transverse division of the centromere in a manner similar to that believed to happen at meiosis, the sectorial plants should show an asymmetry produced by the marked differences in appearance and texture between diploid and secondary tissues. In no variegated plant was tissue characteristic of the secondary found. Secondary chromosomes may arise in somatic cells but no evidence that they do has been obtained. The simple explanation of the transverse division of the centromere will not account for the origin of the diminutive chromosomes. It must be admitted that the nature of these structural changes is unknown but that they are a consequence of the terminal position of the centromere can be argued with some reasonableness. The secondary chromosome is a direct product of the telocentric chromosome but has a median rather than a terminal centromere. If the instability of the telocentric chromosome is due to some factor other than the unusual position of its centromere it would be expected that the secondary chromosome would also be unstable. However, nearly 200 secondary trisomes have been obtained during the course of these studies and no evidence of instability of the secondary chromosome, exhibited either as asymmetrical sectors of growth or variegation, has been found. It is the writer's experience, and he understands also of other maize students, that sectors due to loss or somatic segregation are rarely found in the sporophyte. This is true of trisomic as well as disomic plants. Apparently a chromosome with an interstitial centromere is more stable than one with a terminal centromere.

THE STRUCTURE OF THE CENTROMERE

The centromere appears at pachytene in maize chromosomes stained with aceto-carmine as a simple body with a homogeneous, translucent appearance. There is no suggestion of the compound nature which SCHRADER found for the centromeric region of Amphiuma. In Amphiuma the centromere is a compound body composed of the commissural cup and spindle spherule. The spindle spherule is connected with the half spindle component. Presumably this is a function reserved for the spherule and in case of its loss the commissural region would be unable to form a half spindle fiber. It would seem that the centromeric region of a maize chromosome lacks this specialization of its component parts because MCCLINTOCK (1932, 1938) found that both parts of a fractured centromere were able to function in a normal manner. NEBEL (1939) believes that the centromere is a compound body consisting of three parts: a central achromatic body, the chromatic kinetic bodies (equivalent to spindle spherules), and the chromatic connecting chromomeres of the chromonemata with the achromatic body. If the centromere is broken he assumes that the kinetic body will be regenerated by that part of the achromatic body not retaining it after breakage. In the case of the maize contromere it is difficult to determine the formation or loss of an invisible body. It seems probable that in maize there is no differentiation of the centromeric region into recognizable structures having specialized duties but that on the other hand any part of the centromere region, providing it is not attached to an inordinately large piece of chromatin, is able to function normally. In this connection it should be noted that MCCLINTOCK found that each part of a fractured nucleolar-organizer body was able to function.

DARLINGTON (1939) from a consideration of the transverse division of the centromere of unpaired chromosomes reached certain conclusions concerning its internal structure. He concluded that it possesses a dual nature consisting of a fluid and a fibrous element. The fibrous elements or centrogenes normally control the plane of division or 'explosion' of the fluid element. Since the fibrous elements lie across the centromere the fluid will usually divide in the plane of division of the centrogenes. DARLINGTON accounts for the observed misdivision of the centromeres of univalent chromosomes by assuming that the centrogenes apparently divide after the chromonemata and misdivision is due to their failing (exceptionally) to divide in time for the explosion of the centric fluid which is precocious in univalents at meiosis. DARLINGTON'S conclusions regarding the internal structure of the centromere are admittedly speculative, but it seems to the writer that he is justified in assuming some internal organization within the centromere to account for its normally orderly longitudinal division. Whether or not his conception of the cause of misdivision of the centro-

mere is correct there is no doubt that misdivision does occur since it has been observed cytologically by DARLINGTON, KOLLER, and UPCOTT in addition to the evidence presented in this paper on the genesis of the secondary chromosome.

In considering the ways by which the secondary chromosome could have arisen from the telocentric chromosome, it was suggested that a terminal centromere might be 'sticky' and would occasionally become attached to another terminal centromere especially if they were in close proximity for a considerable time. That is, if two telocentric chromosomes were in the same cell their centromeres might fuse to form a metacentric chromosome. Following RANDOLPH'S (1932) technique, root tips of seedlings hyperploid for the telocentric chromosome were submerged in hot water to induce doubling of the chromosomes. After treatment the root tips were fixed and sectioned. A dozen clear polar views at metaphase of cells with the double number of chromosomes were found and in each the two telocentric chromosomes although lying parallel to one another were clearly separate. Though the heat treatment produced a restitution nucleus with double the number of chromosomes, the terminal centromeres of the two telocentric chromosomes did not fuse after lying in juxtaposition for some hours. These observations are of such a fragmentary nature as to merit little weight but they indicate that the formation of the secondary chromosome occurs when the centromere of the telocentric chromosome has misdivided and not from 'unsaturation' of terminal centromeres.

LEVAN (1938) believes that the division of the centromere is delayed by the alkaloid colchicine. When root tips were treated with colchicine he found at metaphase what he describes as c-pairs formed by the attachment of the two daughter chromosomes to their undivided centromere. The inactivation of the spindle apparatus produced by colchicine is believed to be connected with a delay in the division of the centromere. (This is in agreement with SCHRADER'S and DARLINGTON'S conception of the centromere as playing an important rôle in the development of the spindle.) After a time the centromere divides and the two daughter chromosomes come to lie free from each other but in parallel alignment. Since DARLINGTON believes that the misdivision of the centromere of unpaired chromosomes at meiosis is due to the failure of the centrogenes to divide in time for the explosion of the centric fluid, it seemed possible that a delayed division of the centromere of the telocentric chromosome produced by colchicine treatment might invariably result in the formation of the secondary chromosome. Healthy root tips of plants hyperploid for the telocentric chromosome were submerged in an 0.2 percent aqueous solution of colchicine for one hour. Twenty-four hours later the material was fixed and sectioned. A number of clear figures were found in which doubling had oc-

curred but in no case were the two telocentric chromosomes attached at the centromeric region. More extended observations of both colchicine and heat treated material might have shown an occasional secondary chromosome but there is reason to doubt if either treatment would be effective. Misdivision of the centromere of univalent chromosomes occurs either at or immediately after the meiotic divisions. The centromere of a univalent is, however, at this time in a pecularian situation compared to the paired centromeres of a bivalent and its misdivision results from an aberrant precocious attempt to divide one mitosis in advance of the usual time. In the colchicine and heat-treated material all of the chromosomes are subject to the same forces concomitantly and there is no more delay in the division of the centromere of the telocentric chromosome than of the other centromeres of the chrosome complement.

ON THE ORIGIN OF SECONDARY TRISOMES

Secondary trisomes were first reported by BELLING and BLAKESLEE (1924) in Datura. Since each chromosome is two-armed and the secondary chromosome consists of two homologous arms incorporated into a single chromosome there are two possible secondary trisomes for each chromosome. In Datura all 12 of the possible primary types have been found but only 14 of the 24 secondaries have been discovered (BLAKESLEE and AVERY 1938). In a number of other plants including maize and *Nicotiana sylvestris* all or nearly all of the primary types have been isolated. Secondaries have been rarely reported. The writer (1933a) described a secondary for chromosome 5 in maize and one has been reported by PHILP and HUSKINS (1931) in Matthiola. Recently, GOODSPEED and AVERY (1939) believed they had found several secondary trisomes in *Nicotiana sylvestris* but their classification was based on the appearance of the plants and not on cytological examination so final judgment must be withheld concerning the true nature of their supposed secondaries.

BELLING and BLAKESLEE (1924) suggested that the secondaries originated from a reversed synapsis of two homologous chromosomes and that a crossover occurred at the only place where homologous parts were together which would be the centromere. This hypothesis can be rejected as improbable. BLAKESLEE and AVERY (1938) suggest "that unequal crossing over between parallel sister strands in such a way as to retain spindle attachment points for each newly organized chromosome which has been formed by joining together by their broken ends the two similar halves of the strands affected" might account for the origin of the secondaries. However, the results reported here and by the writer in 1938 as well as the cytological observations by KOLLER, UPCOTT and DARLINGTON make it probable that the secondaries arise through transverse division of the

centromere. While only in the maize secondary has it been established that the secondary chromosome is a strict isochromosome it seems probable that the others are also of a similar structure and have all arisen through misdivision of the centromere. Secondaries might arise directly from an unpaired chromosome by misdivision of its centromere as has been observed cytologically by the investigators mentioned above, or from misdivision of a telocentric chromosome as reported in this paper. The low frequency with which secondary trisomes arise from the telocentric chromosome makes it likely that in many instances the secondaries arise directly from the misdivision of unpaired atelocentric chromosomes although a telocentric chromosome is a potential source of secondaries. If, in organisms with no telocentric chromosomes in the normal complement, secondaries come only from telocentric chromosomes, their frequency would be the product of the probability of a telocentric fragment arising and the probability that once having arisen it would be transformed into a secondary chromosome. There are no data available from which the correlation of the frequency with which telocentric chromosomes arise through misdivision or other causes and the frequency of secondary trisomes can be determined. Since both telocentric chromosomes and isochromosomes were observed by KOLLER, UPCOTT and DARLINGTON as products of the misdivision of the centromere, it is not likely that all secondaries come progressively from telocentric chromosomes followed by misdivision of the centromere. Judging from the data reported in this paper for the maize telocentric chromosome the misdivision of its centromere is a relatively rare event. However, in considering the origin of secondary or isochromosomes it is of interest to note that recently DARLINGTON (1940) followed the behavior of telocentric chromosomes arising through misdivision of the centromere at meiosis in the first microspore division. He states that "Following misdivision of the centromere at meiosis in diploid and triploid Fritillaria new telocentric chromosomes are formed whose broken ends rejoin within the centromere. This type of chromosome is delayed at metaphase and anaphase in the pollen grain mitosis. It may then either break again at the centromere or pass without separation to the pole as a new isochromosome." It is not known whether or not this delayed division of the Fritillaria telocentrics in the pollen grain division also exists in the sporophytic divisions of the following generation. In the case of the maize telocentric reported in this paper there is good reason to believe that the type of misdivision occurring in the gametophyte division does not happen in the somatic division of the sporophyte.

The data compiled by BLAKESLEE and AVERY (1938) on the frequency of secondaries from diploids and related primaries are in agreement with DARLINGTON'S thesis that misdivision of the centromere of univalent chro-

mosomes gives rise to secondaries or isochromosomes. In a trisomic plant one of the three homologous chromosomes is often unpaired which is precisely the condition favoring misdivision. Actually, the Datura workers found that the secondaries were thrown by related primaries 14 times as frequently as by diploids.

The secondary chromosome formed by the misdivision of the telocentric chromosome has two identical arms which is true of the attached X's in *Drosophila melanogaster*. It is doubtful, however, if the attached X's arose by a misdivision of the centromere, although such an origin is a possibility, since L. V. MORGAN (1938) has shown that two X chromosomes may become attached by replacement through crossing over of the two arms of a Y chromosome by X's.

HÅKANSSON (1932) reported a chromosome in Triticum with like ends. While he believes it arose through crossing over in a duplicated segment and its two arms therefore not wholly equivalent, it is possible that it is a true isochromosome and arose through misdivision of the centromere. LOVE (1939) reported ring univalents in Triticum which may be isochromosomes. HUSKINS and SPIER (1934) and LOVE (1938) have reported a chromosome in Triticum with a terminal centromere due to the loss of one arm. It is of some interest to know if these telocentric chromosomes will give rise to isochromosomes. BLAKESLEE and AVERY (1938) state that a telocentric chromosome in Datura consists of the ·11 part of the 11·12 chromosome. If this chromosome has a truly terminal centromere it should form an occasional 11·11 secondary.

RANDOLPH (1928a) has described a type of supernumerary chromosome in maize known as the B-type. MCCLINTOCK (1933) found that the centromeres of the B-types were terminal although DARLINGTON (1937) believes them to be sub-terminal. DARLINGTON'S conclusions were drawn from a study of somatic metaphase plates where he observed a constriction near one end which he interpreted to be the centric constriction. MCCLINTOCK, however, studied the pachytene stage where a much clearer picture of the morphology is obtainable and her published photograph of two paired B-types shows a terminal centromere. While it is possible that there are different kinds of B-types and that those which DARLINGTON studied possessed sub-terminal centromeres, it is not unreasonable to suppose that the constriction observed by DARLINGTON marks the junction of the euchromatin and the deeply staining pycnotic bodies (heterochromatin?) so clearly seen in the pachytene chromosome. While there is some dispute concerning the location of the centromere of the B-type chromosome, if we accept MCCLINTOCK'S findings, as the writer does, it is of some interest that RANDOLPH (1928b and unpublished) has found a series of diminutive chromosomes. All of them probably descended by fragmentation from an

original B-type since LONGLEY (1938) found that a diminutive chromosome frequently synapsed with B-types. This behavior of the B-type corresponds to the fragmentation of the telocentric chromosome reported in this paper and it is not improbable that in both instances the unstability is due to the terminal location of the centromere.

The data reported in this paper on the behavior of the telocentric chromosome leave no doubt that this chromosome is unstable. It gives rise to an isochromosome through misdivision of its centromere and it was also found to undergo structural changes in somatic divisions leading to loss or diminution in size. The mechanism of the latter changes is unknown but the greater frequency of their occurrence in the telocentric chromosome makes it probable that they are a result of the terminal position of the centromere.

S. NAWASCHIN in 1916 declared that no chromosome in the normal complement of any organism possessed a terminal centromere. This is true for plants and may hold for animals. If the behavior of all terminal centromeres is similar to the one reported in this paper the absence of telocentric chromosomes is understandable because the unstability of terminal centromeres would lead to the elimination of chromosomes possessing them.

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During the course of the four years in which the data reported here were obtained it was necessary to determine cytologically the chromosome constitution of literally hundreds of plants in order to insure that the visual classification of the various chromosome types were accurate. Without the efficient help of VIRGINIA H. RHOADES this would have been too arduous a task to have been accomplished and the writer wishes to express his appreciation of her invaluable assistance.

SUMMARY

Maize plants hyperploid for a telocentric chromosome consisting of the short arm of chromosome 5 produce an occasional secondary trisome. The supernumerary chromosome of the secondary trisomes consists of two short arms of chromosome 5 attached to a median centromere. It was shown through the use of mutant genes lying in the telocentric chromosome that it was involved in the formation of the secondary chromosome. The frequency with which secondary trisomes were found when plants hyperploid for the telocentric chromosome were used as the pollen parents was 0.46 percent, while their frequency was only 0.22 percent when the same plants were used as female parents. Pollen hyperploid for either one or two short arms of chromosome 5 rarely functions successfully in competition with haploid grains. It is suggested, therefore, that the secondary chromo-

some arises at meiosis from a transverse division of the centromere of the telocentric chromosome, and that it is occasionally incorporated into the generative nucleus during the first microspore division. The vegetative nucleus would be haploid and pollen tube growth normal but the two sperm would transmit the secondary chromosome.

Data have been obtained which indicate that the telocentric chromosome undergoes structural changes in somatic cells. The production of secondary or isochromosomes at meiosis from the telocentric chromosome and its loss and modification in somatic tissue show that a terminal centromere is unstable. Such a telocentric chromosome would tend to be eliminated by natural selection. This instability may apply to all telocentric chromosomes and account for the fact that telocentric chromosomes are rarely, if ever, found in the normal chromosome complement of any organism.

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TABLE 2

Summary of the data on progenies obtained using plants hyperploid for the telocentric chromosome as male and as female parents.

Type of cross: *bm* ——O- - - - - - / *bm* ——O- - - - - - × *Bm* ——O / *bm* ——O- - - - - - / *bm* ——O- - - - - - and reciprocal

CHROMOSOMAL CONSTITUTION	OFFSPRING OBTAINED WHEN THE HYPERPLOID PLANTS WERE USED AS THE PARENTS INDICATED			
	MALE PARENT		FEMALE PARENT	
	NUMBER	PERCENT	NUMBER	PERCENT
Bm ——O- - - - - - *bm* ——O- - - - - -	18	0.25	10	0.18
bm ——O- - - - - - *bm* ——O- - - - - -	7166	98.91	3738	67.68
Bm ——O *bm* ——O- - - - - - *bm* ——O- - - - - -	37	0.51	1671	30.26
bm ——O *bm* ——O- - - - - - *bm* ——O- - - - - -	0	0.00	5	0.09
Bm ——O- - - - - - *bm* ——O- - - - - - *bm* ——O- - - - - -	0	0.00	0	0.00
bm ——O- - - - - - *bm* ——O- - - - - - *bm* ——O- - - - - -	2	0.03	90	1.63
Bm ——O—— *Bm* *bm* ——O- - - - - - *bm* ——O- - - - - -	21	0.29	9	0.16
bm ——O—— *bm* *bm* ——O- - - - - - *bm* ——O- - - - - -	1	0.01	0	0.00
	7245	*100.00*	5523	*100.00*

TABLE 3

Summary of the data on progenies obtained using plants hyperploid for the telocentric chromosome as male and as female parents.

Type of cross: $\frac{a2}{}$——O- - - - - - / $\frac{a2}{}$——O- - - - - - × $\frac{A2}{}$——O / $\frac{a2}{}$——O- - - - - - / $\frac{a2}{}$——O- - - - - - *and reciprocal*

CHROMOSOMAL CONSTITUTION	OFFSPRING OBTAINED WHEN THE HYPERPLOID PLANTS WERE USED AS THE PARENTS INDICATED			
	MALE PARENT		FEMALE PARENT	
	NUMBER	PERCENT	NUMBER	PERCENT
A ——O- - - - - - a ——O- - - - - -	95	1.74	46	0.82
a ——O- - - - - - a ——O- - - - - -	5303	97.30	4092	73.00
A ——O a ——O- - - - - - a ——O- - - - - -	23	0.42	1385	24.71
A ——O A ——O- - - - - - a ——O- - - - - -	0	0.00	1	0.02
a ——O a ——O- - - - - - a ——O- - - - - -	2	0.04	25	0.45
A ——O- - - - - - a ——O- - - - - - a ——O- - - - - -	0	0.00	0	0.00
a ——O- - - - - - a ——O- - - - - - a ——O- - - - - -	1	0.02	48	0.86
A ——O—— A a ——O- - - - - - a ——O- - - - - -	26	0.48	8	0.14
a ——O—— a a ——O- - - - - - a ——O- - - - - -	0	0.00	0	0.00
	5450	100.00	5605	100.00

TABLE 4

Summary of data when $\frac{Bm}{}$————O / $\frac{Bm}{}$————O- - - - - - / $\frac{bm}{}$————O- - - - - - *individuals were used as the male in backcrosses to diploid bm plants. The phenotypes of the different chromosomal classes are in the second column.*

CHROMOSOMAL CONSTITUTION	PHENOTYPE	OFFSPRING OBTAINED	
		NUMBER	PERCENT
————O- - - - - - ————O- - - - - -	*Bm*	2596	48.06
————O- - - - - - ————O- - - - - -	*bm*	2755	51.00
————O ————O- - - - - - ————O- - - - - -	*Bm*	22	0.40
————O ————O- - - - - - ————O- - - - - -	*bm*	0	0.00
————O- - - - - - ————O- - - - - - ————O- - - - - -	*Bm*	0	0.00
————O- - - - - - ————O- - - - - - ————O- - - - - -	*bm*	0	0.00
————O———— ————O- - - - - - ————O- - - - - -	*Bm*	29	0.54
————O———— ————O- - - - - - ————O- - - - - -	*bm*	0	0.00
		5402	100.000

TABLE 5

Summary of data when

A2 Bm
————————O

A2 bm   *Pr*
————————O- - - - - - -

a2 bm   *pr*
————————O- - - - - - -

individuals were used as the female in back-crosses to diploid a2 bm pr plants.

CHROMOSOMAL CONSTITUTION	PHENOTYPE	NUMBER OF OFFSPRING
————O- - - - - - - ————O- - - - - - -	*a bm*	673
	a Bm	0
	A Bm Pr	2
	A Bm pr	1
	A bm Pr	471
	A bm pr	199
————O ————O- - - - - - - ————O- - - - - - -	*a bm*	0
	a Bm	2
	A Bm Pr	278
	A Bm pr	350
	A bm Pr	3
	A bm pr	0
————O- - - - - - - ————O- - - - - - - ————O- - - - - - -	*a bm*	0
	a Bm	0
	A Bm Pr	0
	A Bm pr	0
	A bm Pr	50
	A bm pr	4
————O———— ————O- - - - - - - ————O- - - - - - -	*a bm*	0
	a Bm	0
	A Bm Pr	2
	A Bm pr	2
	A bm Pr	0
	A bm pr	0

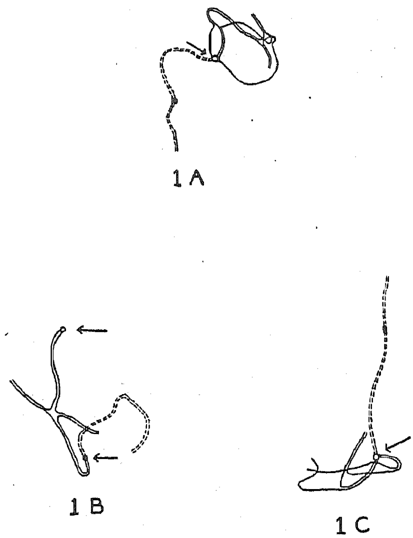
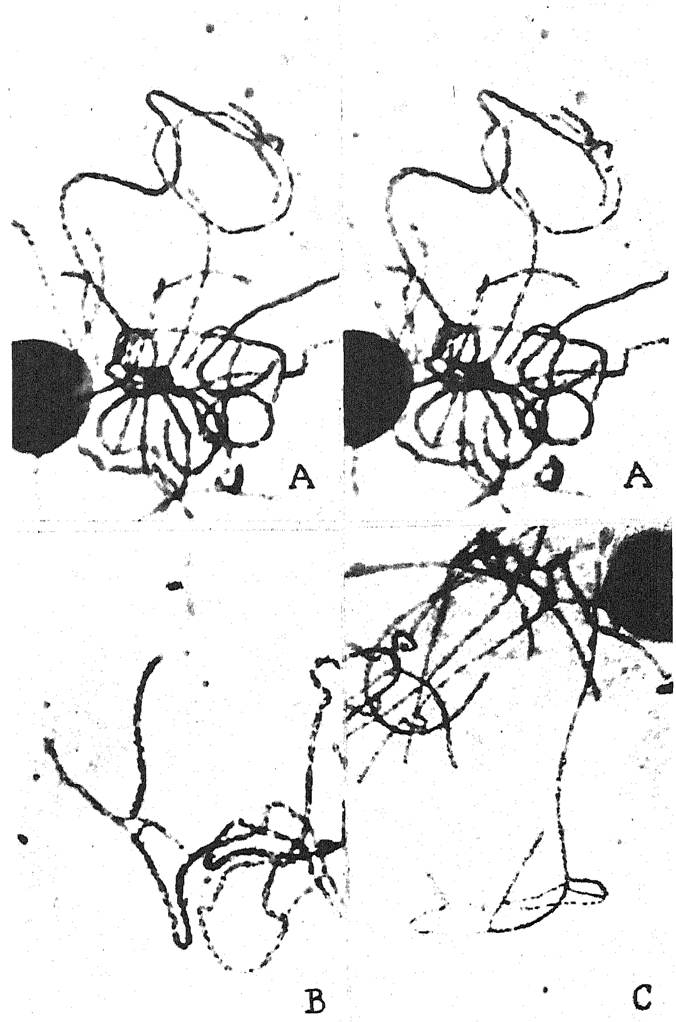
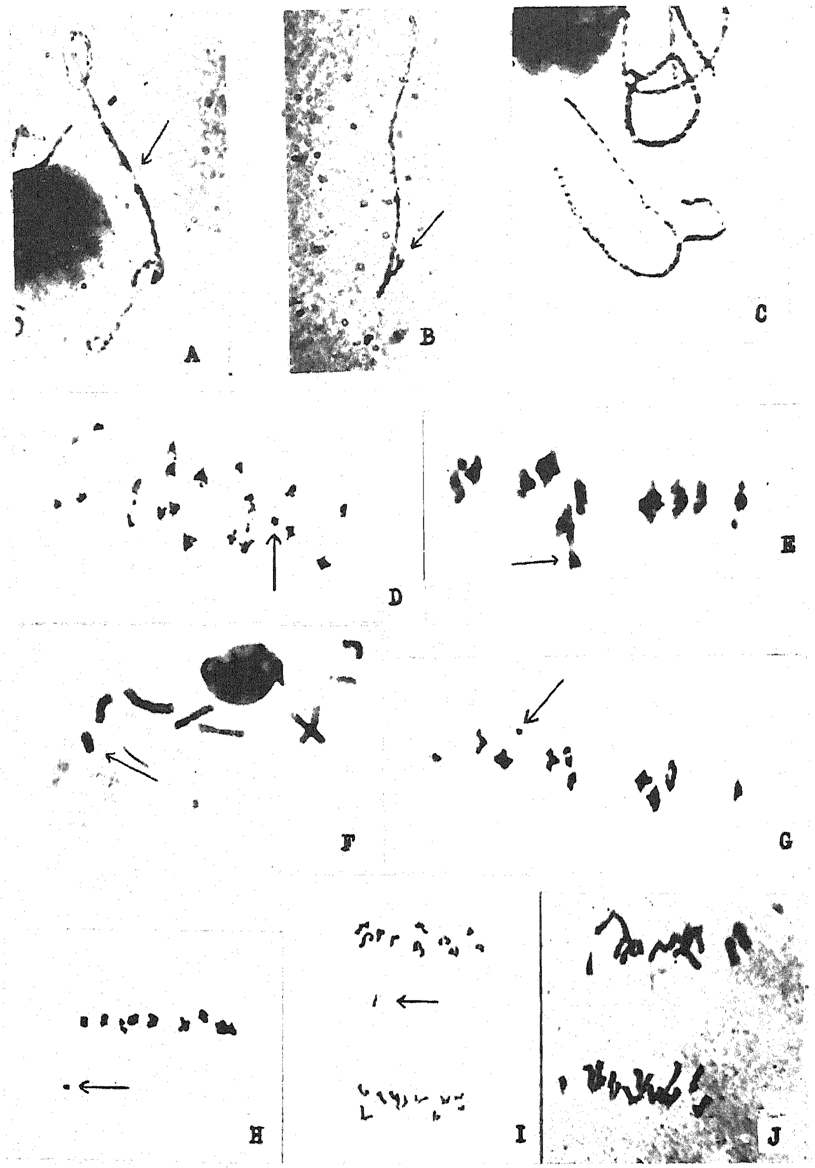


FIGURE 2.—Diagrammatic sketches of the pachytene configurations shown in Plate 1. The centromeres are represented by bulges and are indicated by arrows. The long arms of chromosome 5 are shown by broken lines while the short arms are indicated by solid lines. Figure 1 A corresponds to figure A, Plate 1, figure 1 B to figure B, and figure 1 C to figure C in Plate 1.

EXPLANATION OF PLATE 1

Figures A, B and C show synapsis at pachytene between the secondary chromosomes and the two normal chromosomes 5. The two photomicrographs of figure A are at different levels. Figure B is from a secondary trisome that arose in a stock disomic for chromosome 5 (RHOADES 1933a). The more intimate pairing seen in figure B is not a characteristic difference between this secondary trisome and those arising from the telocentric chromosome. See figure 2 for interpretation.


A
A
B
C


A
B
C
D
E
F
G
H
I
J

EXPLANATION OF PLATE 2

FIGURE A.—Photomicrograph of two chromosomes 5 paired at pachytene. The centromere is indicated by arrow.

FIGURE B.—Photomicrograph at pachytene of unpaired telocentric chromosome. The equational split is evident at the distal end. The terminal centromere is indicated by the arrow and was clearly terminal.

FIGURE C.—Photomicrograph at pachytene of telocentric chromosome with its centromere stuck to the centromeres of two paired chromosomes 10. The distal end of the telocentric has a foldback, that is, it is paired hon-homologously in this figure. There is no suggestion that the telocentric chromosome is two-armed.

FIGURE D.—Early anaphase showing disjunction of the paired homologues while the unpaired telocentric chromosome is still on the plate. In late anaphase it may separate equationally and its two chromatids (daughter univalents) migrate to different poles.

FIGURE E.—Metaphase I showing trivalent composed of telocentric and two chromosomes 5. The orientation of the telocentric chromosome, indicated by arrow, is such that it will disjoin with a normal chromosome 5.

FIGURE F.—Late prophase in microspore hyperploid for the telocentric chromosome which is indicated by arrow.

FIGURE G.—Metaphase I showing 10 bivalents and unpaired telocentric chromosome which has congressed on the spindle.

FIGURE H.—Metaphase II with 10 dyads on the equatorial plate and a daughter univalent, arising from the equational separation of the telocentric chromosome in anaphase I, lying off the plate.

FIGURE I.—Anaphase II with 10 monads passing to each pole while a daughter univalent lags. Origin of daughter univalent same as in figure H.

FIGURE J.—Anaphase II with 11 monads passing to each pole. In the preceding anaphase the telocentric chromosome was a member of a trivalent group and underwent a reductional division. The two daughter univalents of the telocentric chromosome are rod-shaped because of their terminal centromeres while the other monads are V- or J-shaped.

Explanation of Plate 3

Figure A.—Metaphase I showing that the centromere is divided at its poleward tip. Each chromatid will form its own half-spindle component but the bulk of the centromere does not appear to be divided.

Figure B.—Anaphase I showing centromeres divided at poleward tips. As in figure A the bulk of the centromere does not appear divided.

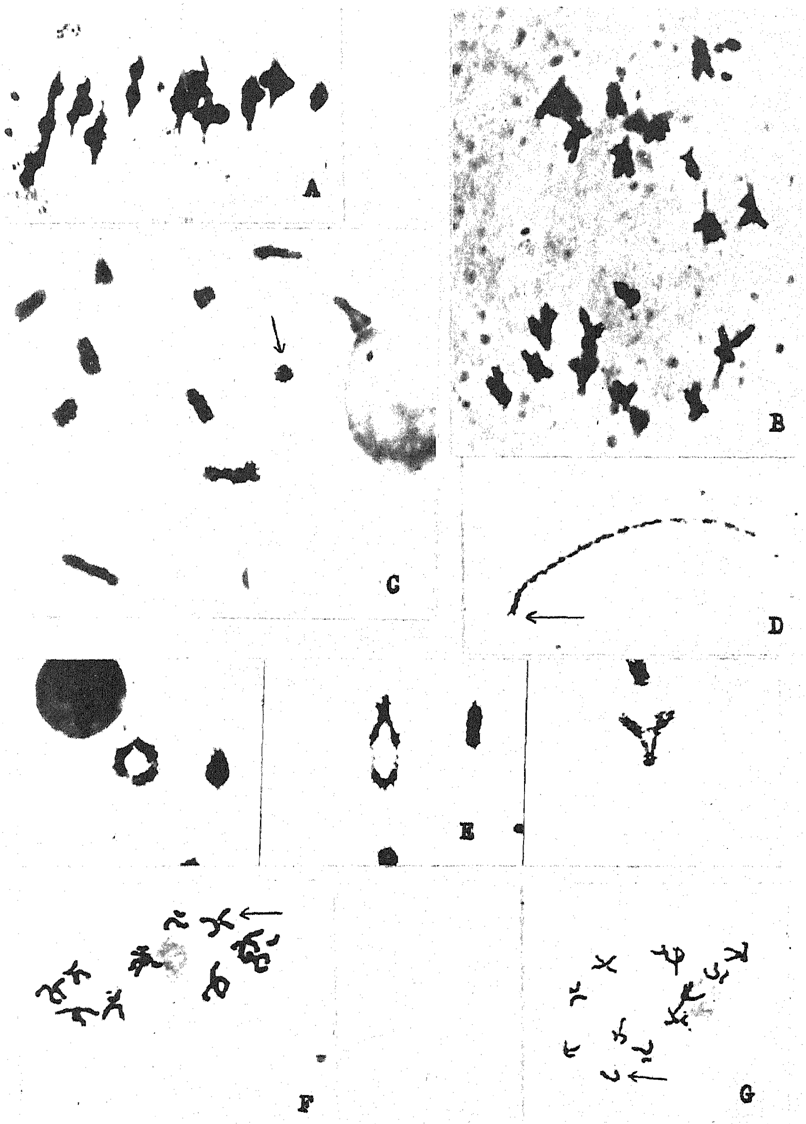
Figure C.—Diakinesis in secondary trisome with the secondary chromosome present as a ring of 1. This configuration results from chiasma formation between its two homologous arms.

Figure D.—Pachytene stage showing pairing of the two identical arms of secondary chromosome. The median centromere (see arrow) has a terminal position because of the synapsis of the two homologous arms. This configuration will give a ring of 1 at diakinesis.

Figure E.—Diakinesis in secondary trisome. Three ring configurations produced by pairing of secondary chromosome with the two normal chromosomes 5. These rings of 3 are produced by pachytene associations shown in Plate 1. In the leftmost ring terminalization is complete while it is only partially so in the middle figure and in the rightmost figure there has been little if any movement of the chiasmata.

Figure F.—Prophase II with the secondary chromosome indicated by arrow. Since the two arms of this chromosome are alike the dyad appears as an X-shaped element with all four arms of the X of equal length. In the dyad to the left of the secondary the two short arms appear continuous as do the two long arms. The unstained centromere lies between the two chromatids at the center of the X. The rod-shaped body to the right is a B-type daughter univalent.

Figure G.—Prophase II showing the two chromatids of a telocentric chromosome. Since the two chromatids appear continuous the centromere must be forced to one side at this stage. If the centromere was not strictly terminal an X-shaped figure would be produced at prophase II as is the case for the rest of the chromosome complement.


A
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G

THE TEMPERATURE RESPONSES OF FLIES WITH THE DEFICIENCY VESTIGIAL-DEPILATE IN DROSOPHILA MELANOGASTER[1]

MORRIS HENRY HARNLY
Washington Square College of Arts and Sciences, New York University

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INTRODUCTION

THE effects of temperature on a number of the genotypes involving the vestigial locus of *D. melanogaster* have been reported in earlier papers (HARNLY 1930a, b, 1932, 1933, 1936; HARNLY and HARNLY 1935, 1936). During these investigations it became evident that the critical temperatures for a marked increase in wing size vary with the genotype (there may be two critical points for one genotype) and that the phenotype of an individual is dependent on both its genotype and the temperature experienced by it during a critical period of development. Consequently, a given phenotype may be produced at different temperatures by a number of genotypes. For example, the "strap" wing phenotype was produced by the strap allele of vestigial at 25°C but may be produced by vestigial at 31° and 32°, by dimorphous vestigial at 25° and 29°, by vestigial-pennant/vestigial at 26° and 28°, by vestigial-Depilate/vestigial-pennant at 16°, etc. An hypothesis has been proposed for a definite pattern of wing form in replacement and development. This hypothesis is based on the data covering known gene substitutions at one temperature, a single genotype at different temperatures, and transfers between two temperatures for each individual during its development. The duration of the temperature-effective-period of wing development, the rate of the growth processes involving the wings during this period, and the general growth rate of the individual vary independently of each other through the viable temperature range. It has been possible to distinguish between the effect of the gene on growth and on differentiation through the independent changes in size, and the form of the wings at different temperatures. It has not been possible to distinguish clearly between the powers or effects of the two alleles in the heterozygote since the action of each alone was known only in the double dose of the homozygote. The work reported here and in a paper following is an attempt to determine something of the powers of alleles at the vestigial locus in the haplo-condition and so determine their respective rôles in the heterozygote. The experiments were performed several years ago immediately after the work on vestigial, dimorphos vestigial, pennant, and

[1] The author wishes to express his thanks to MISS RUTH RONES for technical assistance in this work.

pennant/vestigial while the stocks were isogenic from repeated intercrosses.

DESCRIPTION OF DEPILATE

Depilate (vg^D), formerly called $vg^{De\ 31\ a\ 22}$ by BRIDGES, removes a zone of microchaetae from the thorax, and hairs and bristles from the legs. It has been found to be a deficiency for vestigial (BRIDGES), for 1C (CURRY), and for scabrous (IVES). BRIDGES has found it shows a salivary chromosome deficiency for 49 C 1–49 E 2 inclusive. Classification and viability are excellent but it is lethal when homozygous. There may be a slight reduction of crossing over (D.I.S. number 4, Sept. 1935).

STOCKS AND METHODS

Vestigial-Depilate/Upturned flies were obtained from Dr. DEMEREC. These were crossed with a wild strain that had been repeatedly backcrossed to the inbred vestigial stock used in the temperature experiments mentioned above. In the F_1 and succeeding generations the $vg^D/+$ females were backcrossed to males of this special wild type. This produced a Depilate stock identical in its gene complex with the wild type, vestigial, dimorphos vestigial, and vestigial-pennant stocks which had been interbred and used in my work with temperature, differing from them only by the short deficiency in the region of the vestigial locus. Consequently, all my previous work with the vestigial locus should be comparable with the results obtained from mating these stocks to this specially bred Depilate line.

In the first series of tests, $vg^D/+$ females of this prepared line were mated to males of the special wild-type stock. Eight pairs per vial were allowed a $1\frac{1}{2}$ hour egg-laying period at 25°C. They were then repeatedly transferred to new vials for similar periods. The number of offspring per vial was below the point at which crowding influences the wing size and form. All the vials remained at 25° for 24 hours. At that time practically all viable eggs had hatched (HARNLY 1929). The vials were then distributed so that equal numbers of the offspring of each set of parents were placed in incubators set at 16°, 20°, 24°, 28°, 30°, and 32°C. All further development took place at the temperature specified with the exception of those individuals placed at 32°. This temperature is lethal for total development and the vials placed at 32° were returned to 25° after 120 hours (five days) of development for the completion of pupation and emergence of the adults. There were two types of offspring in these trials, $vg^D/+$ and $+/+$. They could be separated readily by the absence of microchaetae on the thorax of the $vg^D/+$ flies. In the second series of trials $vg^D/+$ females were mated to males of the inbred vestigial stock.

The handling of these trials was identical with that of the first series described above. The offspring were of two types: vg^D/vg $+/vg$ and could be separated by both the absence of microchaetae on the thorax of the vg^D/vg flies and the difference in wing size.

The air temperature variation within the incubators was ±0.05° and the incubators were kept in a cold room (10°) which varied less than 1° in temperature. The accuracy of the incubators, the temperature control mechanism, the food and vials used, and the method of measuring the wings have all been discussed elsewhere (HARNLY 1936). Measurements were made of the right wing of 60 males and 60 females at each temperature for the genotypes $vg^D/+$ and vg^D/vg.

TEMPERATURE EFFECTS ON VESTIGIAL-DEPILATE/WILD

The mean wing lengths and areas of $vg^D/+$ flies reared at the temperatures indicated are recorded in tables 1 and 2. The wings of such flies vary

TABLE 1

Mean wing length in mm of vestigial-Depilate/wild flies, 3 trials.

°C	MALES		FEMALES	
	LENGTH ± P. E.	σ	LENGTH ± P. E.	σ
16	2.47±0.006	0.07	2.73±0.009	0.11
20	2.40±0.007	0.07	2.70±0.009	0.10
24	2.22±0.007	0.08	2.51±0.009	0.10
28	2.09±0.005	0.05	2.35±0.009	0.10
30	2.00±0.005	0.06	2.27±0.007	0.08
32	2.04±0.007	0.08	2.32±0.009	0.10

in size inversely with the temperature for both the males and the females. The wings of the females are regularly larger than those of the males. The decrease in wing size from 16° to 30° is the same for both sexes, the mean

TABLE 2

Mean wing area in sq. mm of vestigial-Depilate/wild flies, 3 trials.

°C.	MALES		FEMALES	
	AREA ± P. E.	σ	AREA ± P. E.	σ
16	1.64±0.009	0.11	1.91±0.012	0.14
20	1.55±0.007	0.09	1.95±0.010	0.12
24	1.34±0.007	0.08	1.67±0.011	0.12
28	1.17±0.005	0.06	1.45±0.010	0.12
30	1.08±0.005	0.06	1.34±0.008	0.09
32	1.12±0.007	0.09	1.41±0.010	0.12

length decreasing 0.5 mm and the area 0.6 sq. mm. No profound change in wing phenotype occurs and the post-scutellar bristles and balancers are normal throughout the temperature range examined. The change in slope of the curves for the males and the females between 30° and 32° is probably due either to the marked lengthening of the larval period at 32° (HARNLY 1936) or to a shift in dominance. A shift in dominance of pennant/vestigial has already been observed (HARNLY and HARNLY 1936). Between 20° and 30° the curves are practically straight lines. With the same parentage the adult yield from the vials at 30° ran some 50 percent behind that of the vials at lower temperatures. Equal proportions of +/+ and $vg^D/+$ would be expected from a backcross and were obtained at all the points except 30°. There the frequency of the $vg^D/+$ compared to their +/+ sibs was definitely below expectation. Apparently the deficiency had lowered the lethal point significantly.

TEMPERATURE EFFECTS ON VESTIGIAL-DEPILATE/VESTIGIAL

The mean values for wing length and area of vg^D/vg flies are given in tables 3 and 4. The curves for vg^D/vg give the appearance of very little change in wing size through the 16° range examined in this report. However, a consideration of the data in tables 3 and 4 shows very definite

TABLE 3

Mean wing length in mm of vestigial-Depilate/vestigial flies, 3 trials.

°C	MALES		FEMALES	
	LENGTH ± P. E.	σ	LENGTH ± P. E.	σ
16	0.57±0.011	0.11	0.57±0.008	0.09
20	0.58±0.009	0.10	0.58±0.007	0.07
24	0.57±0.008	0.09	0.65±0.007	0.08
28	0.62±0.008	0.09	0.70±0.006	0.07
30	0.68		0.78	
32	0.71±0.004	0.05	0.76±0.006	0.07

changes in wing size for both sexes. The wings of the males lengthened 25 percent over their lowest mean value and enlarged 56 percent in area; the increases over the lowest mean value for the females are 37 percent in length and 89 percent in area. These total changes are not due to a uniform increase between 16° and 32°. The wings of the males did not lengthen between 16° and 24° but did lengthen regularly between 24° and 32°; the curve being practically linear through that temperature range. The area of the wings increased more or less regularly from 16° to 32°. The wings of the females lengthened only slightly between 16° and 20° but quite definitely above that point, the curve between 20° and 32° being practically

TABLE 4

Mean wing area in sq. mm of vestigial-Depilate/vestigial flies, 3 trials.

°C	MALES		FEMALES	
	AREA ± P. E.	σ	AREA ± P. E.	σ
16	0.09±0.003	0.03	0.09±0.002	0.02
20	0.11±0.003	0.03	0.11±0.002	0.02
24	0.11±0.002	0.03	0.14±0.003	0.03
28	0.12±0.002	0.03	0.16±0.002	0.03
30	0.14		0.16	
32	0.14±0.002	0.02	0.17±0.002	0.02

a straight line. Their wings increase regularly in area from 16° to 32°, the amount being almost constant for each 4° rise in temperature. As in the case of the wings of *vg/vg*, the wings of vg^D/vg (haplo-vestigial) flies vary directly in size but not always proportionately with the temperature. The wings of the females are larger in length and area than those of the males at 24° and all temperatures above that point, a sexual dimorphism found in +/+, +/*vg*, $vg^D/+$ and vg^p/vg^p but not found consistently between 24° and 32° for *vg/vg*. The wings of the vg^D/vg females attain a length at 24° typical for vestigial and the males do likewise between 28° and 30°, but even at 32° neither sex develops wings typically vestigial in area. The balancers (halteres) remain mere vestiges and the post-scutellar bristles point cephalad between 16° and 32° showing none of the changes that occur in these structures of *vg/vg* through this temperature range.

The yield of flies and the proportion of +/*vg* to vg^D/vg were normal for a backcross between 16° and 24° but at 28° the vg^D/vg class was approximately 25 percent of expectation. At 30° only 19 vg^D/vg flies emerged from some 90 vials. The frequency of survival at 28° for vg^D/vg is approximately that of *vg/vg* at 31°. Evidently the lethal point was lowered approximately 3° by this deficiency in the vestigial animals. One cannot depend on the wing values at this temperature because of the very few individuals obtained at 30°. However, after 120 hours of development at 32° followed by transfers to 25° for the completion of development the flies emerged in the usual equal proportions of the two phenotypes from a monohybrid backcross, and the expected yields were obtained. The wing values at 32° are based on satisfactory yield, ratio of types, and number of individuals and can be considered significant. The profound increase in wing size characteristic for *vg/vg* at this point did not take place in the vg^D/vg individuals.

TEMPERATURE AND WING FORM

The wings of the original vestigial-Depilate/Upturned stock are wild-

type in size but show nicks regularly in the distal margin between the second and third, and third and fourth longitudinal veins. Approximately 20 percent of them have one wing with a complete margin and some 7 percent have perfect margins on both wings. If lateral nicks occur at 24° they must be extremely rare as none were noted in over six hundred individuals examined.

In the work reported here wings similar to those just described were developed at all temperatures by the prepared $vg^D/+$ stock and these wings were also phenotypically identical with those previously reported and figured for vg^p/vg^p (figure 3, HARNLY and HARNLY 1936). Again in the case of $vg^D/+$ the phenotype does not vary markedly through the temperature range examined. The only detectable change was in the frequency of nicks on the lateral margins and of wings with perfect margins. The frequency of right wings with one or more nicks in the lateral margins dropped rapidly: males 16°—38 percent, 20°—17 percent, 24°—8 percent, and 28°—3 percent; females 16°—65 percent, 20°—43 percent, 24°—12 percent, and 28°—7 percent. Lateral nicks were not observed in the wings of the males at 30° and 32° and on only one female at 30° with none showing at 32°. Wings with lateral margin nicks and no distal nicks were found only at 16° and occurred five times in 120 observations. The frequency of either a right or a left wing or both wings with perfect margins increased from 15 percent at 16° to 33 percent at 28° for the males, and from 10 percent at 16° to 65 percent at 30° for the females. The disappearance in these flies of lateral margin nicks followed by a marked increase in freedom from distal nicks is further evidence for our hypothesis of pattern in wing development and replacement by gene substitutions. This hypothesis is based on our data from partial development of vestigial at a high temperature with completion at a lower temperature (HARNLY 1936), the phenotypic changes of a single genotype through the viable temperature range (HARNLY and HARNLY 1935, 1936), and the results of MOHR (1932) from allelic substitutions at a single temperature. This hypothesis assumes growth or replacement first distally through the region of the second, third and fourth longitudinal veins, followed by a lateral completion of the wing, the distal margin in the region of the third and fourth longitudinal veins being the last to develop or to be replaced (HARNLY and HARNLY 1935, HARNLY 1936). It must be noted in connection with this hypothesis and the data presented above for $vg^D/+$ that vestigial-pennant developed perfect margins on one or both wings only at 16° and above this point the frequency of wings with defective lateral margins mounted rapidly.

The data from those genotypes producing at least occasional perfect wings make possible the consideration of an interesting question. Are the wing buds of the left and right mesothoracic discs each self-determining,

TABLE 5

Frequency with which both wings are normal (per 1000) in vestigial-Depilate/wild flies.

°C	MALES		FEMALES	
	CHANCE	OBSERVED	CHANCE	OBSERVED
16	7	17	6	50
28	33	33	133	170
30	6	18	228	308
32	3	33	66	133

or are they dependent, at least in part, on materials or events originating outside of the discs? One might assume that if they are self determining the frequency of two perfect wings on the same fly would be that of chance calculated in terms of either of two events happening separately (left, right), or both events happening simultaneously. If they are not self-determining the frequency of both wings perfect might be some other value than that expected by chance. Each occurrence of left normal, right normal, and both wings normal was recorded for all of the $vg^D/+$ flies examined at each temperature in this work. From these data were calculated the frequency by chance and the observed frequency of both wings normal (table 5). Similar calculations on a test of the original vestigial-Depilate/Upturned stock at 24° produced for the males, values of 30 by chance and 73 observed; and for the females, values of 29 by chance and 69 observed. Our earlier data on pennant/pennant at 16° (HARNLY and HARNLY 1936) when calculated gives: males chance 101, observed 180; females chance 100 and observed 171. It is obvious that for at least these three genotypes the frequency of both wings perfect is far greater than is expected by chance. Consequently it is evident that the wing buds are not self determining.

Since they are not self determining there must be some outside factor involved. Some other organ may produce a material essential for wing bud formation which reaches the discs by way of the body fluids. A rather extensive list of organs have been tested by micro-injection with negative results. These tests are being continued.

The difference between the expected by chance and the observed frequency of both wings perfect varies with both the genotype and the temperature. The observed frequency was eight times the frequency by chance at 16° for $vg^D/+$ females but only 1.7 times expectation for vestigial-pennant females at the same temperature. The variation with temperature for a single genotype has been shown above. An investigation along these lines of eight additional genotypes is nearing completion and the results with their implications will be reported in the near future.

The vg^D/vg wings of both sexes at 30° and 32° are vestigial in phenotype, but slightly narrower than those of homozygous vestigial flies reared between 24° and 28°. The progressive decrease in the size of the wings of the vg^D/vg flies with a fall in temperature results in a definite change in phenotype between 32° and 16°. What little is left of the basal region of the wild-type wing in the phenotype known as vestigial, is reduced at lower temperatures in vg^D/vg flies and practically disappears at 20° and 16°. All that remains in extreme cases is the shoulder jutting out from the thorax bearing the vein formed by the fused bases of the I, II, and III longitudinal veins. This vein is surrounded by a thin envelope of wing tissue. A wild-

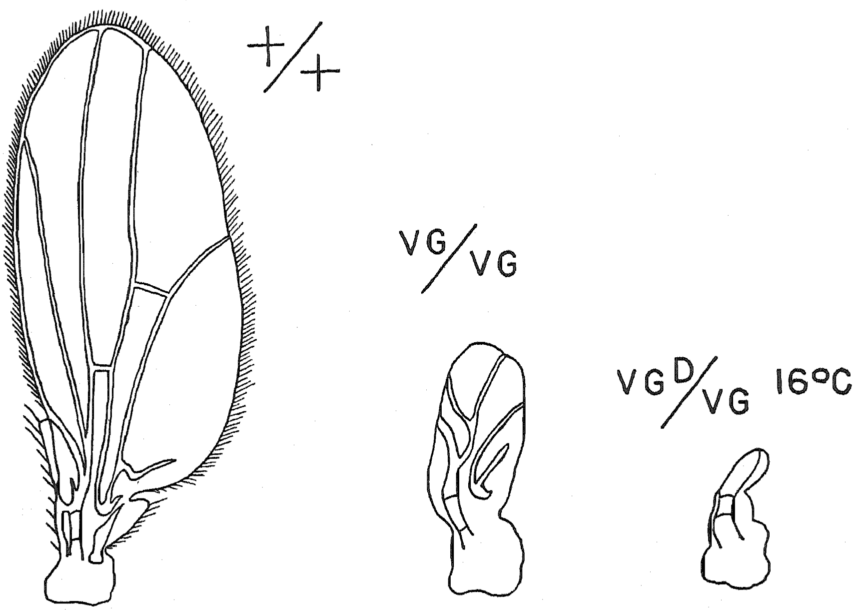

FIGURE 1.—Wings of wild type, vestigial and vg^D/vg flies at same magnification; 16°C.

type wing, a typical vestigial wing, and this rudiment found on vg^D/vg at low temperatures are all drawn at the same magnification in figure 1.

DISCUSSION

STANLEY (1935) has reported a detailed examination of +/+ and +/vg wing length through the viable temperature range. A comparison of his values for these two genotypes with the data reported here for vg^D/+ shows a close agreement for the three genotypes in degree of sexual dimorphism, trend of the curves, values for wing length at each point, and total change in wing length. The modifiers present in his stocks must differ from those in mine and are probably the cause of the slight differences in

our values at some points. Obviously "dominance" in these cases means that one wild-type gene at the vestigial locus (in collaboration with the other normal wing genes) follows the wild type response to temperature and is capable of producing wild-type wings. This is true if the other second chromosome carries either a recessive allele at the vestigial locus or a short deficiency including it. However, $+/vg^D$ (Df [2] vg^D,—bands 49 C 1–49 E) is normal in phenotype while $+/vg^S$ (Df [2] vg^S,—bands 49 C 1 to just to the left of 50 A) has markedly abnormal margins at 25° (MOHR 1932). The normal phenotype and "dominance" depends on the extent of the deficiency and the exact loci absent.

Between 16° and 28° the curves for $vg^D/+$ males and females coincide with those of vestigial-pennant, a regressive mutation from vestigial to a recessive wild phenotype (HARNLY and HARNLY 1936). But at 30° the wings of the homozygous vestigial-pennant males and females are respectively some 0.2 mm and 0.3 mm shorter than those of the male and female $vg^D/+$ flies. In our report on vestigial-pennant it was suggested that there must be a critical temperature for change in wing size between 28° and 30° for this allele. There is no similar sharp inflection between 28° and 30° of the curves from the data presented here for $vg^D/+$ nor in the curves of STANLEY for $+/+$. If the apparent critical point between 28° and 30° for vestigial-pennant is real, then it constitutes an additional difference from the wild type in the mutation from vestigial to a recessive wild-type allele. We have reported previously a number of differences between this recessive wild-type reversion and the dominant wild-type allele (HARNLY and HARNLY 1936).

In the case of the $+/vg$ heterozygote the normal allele together with the other wild-type wing genes produces the normal wings and apparently prevents the inception of the wing-forming processes by the vestigial allele in the heterozygote. This would be the obvious interpretation of the $+/vg$ response when it is compared with that of wild and $vg^D/+$ through the temperature range examined, and one considers the temperature-effective-periods in figure 17 of STANLEY'S (1935) paper. In that figure the termination of the temperature-effective-period of vestigial and the inception of that period for wild barely overlap at 17° and 27°, but the effective-period for $+/vg$ coincides almost exactly with that of the wild homozygote at 17° and again at 27°. Several explanations may be offered for the failure of the vestigial gene in the heterozygote to initiate the processes involved in wing development. It is possible that the wild allele "inhibited" the earlier action of the vestigial gene. At present there is no evidence for such an assumption regarding the wild allele. From figure 1 and tables 3 and 4 in this paper it is obvious that a single vestigial gene produces very little wing material below 28°. The effect on wing size of the single vestigial gene

in the $+/vg$ larvae may have been so small that it was not detectable in the measurements of the wings of the heterozygotes transferred earlier than the beginning of the effective period for the wild-type allele. A third possible explanation is a shift in the time relationships of the activities of the vestigial gene when heterozygous with different alleles and associated with various modifiers. Marked shifts in the critical temperature under such conditions have already been demonstrated (HARNLY and HARNLY 1935, 1936). It may be that from one genotype to another similar changes occur in the time relationships during ontogeny. The detailed discussion and acceptance or rejection of a time relation hypothesis will have to await the appearance of further data on genes at this locus. Data on the temperature-effective-periods and on the development of the wing buds of the mesothoracic discs of several more genotypes will be available in the near future.

The size of the wings of the vg^D/vg and the vestigial flies varies directly with the temperature at which the flies develop. Critical temperatures for a marked increase in the length of the wings have been found for homozygous vestigial males at 30° and for the females at 31° (HARNLY 1930, STANLEY 1931). A comparison of the curves for wing length and area of vestigial with those of vg^D/vg shows one profound difference between the two genotypes in their response to temperature. The very sharp inflection in the curves of vestigial at a high temperature does not occur in the curves for vg^D/vg. There is no significant change in the slope of the wing area curves for these heterozygous males and females between 16° and 32°. The wings of the males do not increase in length between 16° and 24° but do lengthen regularly above that point. The wings of the females do not change between 16° and 20°, lengthening regularly through all higher temperatures. Any increase in length was apparently dependent upon the previous completion of the very basal region of the wing (figure 1). From the data above it is evident that there was no critical temperature for the increase in wing area of the heterozygous flies, the wings enlarging uniformly throughout the temperature range examined. Critical temperatures between 20° and 24° for the females and 24° and 28° for the males for increase in wing length may be assumed from the data and the curves. At present I would rather not call these points critical temperatures. They do not show the profound changes associated in vestigial with a rise of one degree nor do they appear to correspond to the marked lowering of the critical temperature by the introduction of the sex-linked dimorphos gene into the vestigial genotype which we have reported previously (HARNLY and HARNLY 1935). These points of inflection in the curves for length of the wings of vg^D/vg are not conditioned apparently in the same way as those of homozygous vestigial, vestigial-pennant, dimorphos vestigial, or

the heterozygote vestigial-pennant/vestigial. Critical temperatures, as I have used the term to date for these genotypes, implies a marked change in the phenotype at a one degree interval. In terms of the data of the temperature-effective-periods it is interpreted as a marked change in duration, rate, or both of certain developmental processes affecting specific organs or structures and is not accompanied by a proportionate change in the duration of the developmental period of the entire organism. It is the temperature at which a change occurs in the wing determining activities with no change in the general rate and duration of developmental activities of the genotype. In the case of vg^D/vg there is no change in slope of the area curves. The conditioning factor in the length curves for a change in slope seems to be merely the attainment of a certain breadth of wing or amount surrounding the basal vein. Temperature is a secondary factor acting indirectly on length through its direct effect on area where it shows no critical point. This difference between the response of vestigial and vg^D/vg at high temperatures calls for an explanation.

The critical temperature reported by both STANLEY and HARNLY for vestigial may not be due to the vestigial gene. Instead, the temperature response might be controlled by one or more other loci closely associated with the vestigial locus in the second chromosome. The apparent disappearance of the critical temperature for a marked increase in the size of the wings of the vg^D/vg flies would then be due to the absence of other genes in the vestigial-Depilate chromosome close to the deficiency at the vestigial locus. There are certain facts in disagreement with this assumption. The stocks used in the series of papers (1930–36), including the vestigial-Depilate stock, have all been interbred to produce genotypes identical except for the specific genes under examination. Since the crosses reported here were $vg^D/+$ to wild, and to vestigial, the Depilate chromosomes were identical in the two sets of data.

It was noted above that $vg^D/+$ produced curves super-imposable on those of STANLEY for wild through the temperature range examined. The absence of one set of genes in the immediate vicinity of the vestigial locus had caused no change in the temperature response. If the non-appearance of a critical temperature in the vg^D/vg data is attributed to the haplo-condition of genes in the vestigial chromosome then it must be assumed that these genes differ from those in the wild-type chromosome. But this cannot be true because the wild-type and vestigial chromosomes had been carried together in heterozygous females for many generations.

The assumption of genes determining the temperature response being closely linked to the vestigial gene is very weak historically and there are no data to support it. As early as 1918 ROBERTS reported that high temperatures had a profound effect on the expression of homozygous vestigial.

Subsequently sub-lethal or semi-lethal temperatures were used with vestigial in a number of laboratories in routine class experiments to demonstrate the effect of the environment on the expression of the gene. Later on identical critical temperatures were reported in genealogically widely divergent stocks of vestigial flies (HARNLY 1930, STANLEY 1931). If the critical point is determined by a gene linked to vestigial then in twenty years one would have expected that in some stock a crossover or mutation would have occurred resulting either in a stock showing no response to temperature or else one showing a marked effect at some other temperature. In all the stocks of vestigial used such a change has never occurred. Furthermore, PEARL (1928) in his experiments on longevity was unable by repeated outcrosses to dissociate any of the morphological or physiological effects attributed to the vestigial gene. The historical facts support only one assumption—namely, that in the case of vestigial the direct but disproportionate variation in wing size with temperature is a characteristic of the vestigial gene. It has already been demonstrated that the critical point is capable of being either markedly or slightly shifted by different modifying genes (HARNLY and HARNLY 1935, STANLEY 1935). But the breeding methods used and the facts discussed above prevent offering modifying genes as an explanation of the absence of a critical point for wing size at a high temperature in the vestigial-Depilate/vestigial data. A more probable explanation may be found in the facts already known about the vestigial gene and in its relationship to the genotype as a whole.

The presentation of general considerations on gene action during development as determined from temperature studies will appear in the forthcoming paper on vestigial-Depilate/vestigial-pennant. At that time the data on the temperature responses and temperature-effective-periods of several more genotypes will be available to the reader from other papers to appear shortly.

SUMMARY

1. The wing length and wing area of male and female vestigial-Depilate/wild flies varies inversely with the temperature. The wings are practically normal, and the balancers and the post-scutellar bristles are normal throughout the temperature range examined. The mean wing lengths and areas are practically identical with those reported by STANLEY for wild type and wild/vestigial and by us for vestigial-pennant. The deficiency had lowered the lethal temperature.

2. The wing length and wing area of vestigial-Depilate/vestigial flies vary directly with the temperature for both sexes. The wings vary from minute stubs to vestigial in phenotype. The halters and the post-scutellar bristles are not normal from 16° to 32°. The marked increase in wing size

found for vestigial at high temperatures did not occur in vestigial-Depilate /vestigial. The lethal temperature was lowered some 3°.

3. Data presented indicates that the left and right dorsal mesothoracic discs are not self-determining.

4. A small deficiency in the region of the vestigial locus paired with a normal wild-type second chromosome can produce a wild-type wing. A wild genotype heterozygous for a slightly larger deficiency in the same region produces a markedly abnormal wing. The normal phenotype and dominance depend on both the extent of the deficiency and the exact loci absent.

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THE STRUCTURE OF SALIVARY GLAND CHROMOSOMES OF DROSOPHILA MELANOGASTER IN EXCHANGES BETWEEN EUCHROMATIN AND HETEROCHROMATIN

EILEEN SUTTON*

Carnegie Institution of Washington, Cold Spring Harbor, New York

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INTRODUCTION

IN RECENT years SCHULTZ (1938) and PROKOFYEVA-BELGOVSKAYA (1937) have published the results of studies on certain rearrangements in the salivary chromosomes of *Drosophila melanogaster*, in which exchanges of material have occurred between euchromatic and heterochromatic parts of the chromosomes. These authors have both concluded that such rearrangements tend to affect the structure of the bands adjacent to the breaks.

PROKOFYEVA-BELGOVSKAYA (1937) states that "a transfer of any chromosome section to the chromocentral region modifies the structure of that section into a chromocentral structure. . . . Conversely, the removal of chromosome sections of the inert region from the chromocenter (by an inclusion into the active region) brings about a change in the cytological structure of the translocated sections; they become indistinguishable from the active part of the chromosome."

SCHULTZ's results from direct observation and ultra-violet photography do not show a constant modification of bands, but he claims that euchromatic bands transferred to the chromocenter are frequently intensified, showing an increased nucleic acid content. Less frequently the bands nearest to the heterochromatic region are not visible, owing either to their complete loss or to their structure having become indistinguishable from that of the chromocenter; while bands a little further away are more intense than they appear to be in the normal chromosome.

At the suggestion of DR. DEMEREC, a further study of the problem has been made from material at hand in this laboratory. The results are presented here.

MATERIAL

The following rearrangements were studied:

A. Insertions of 3C bands into chromocenter.

1) Dp(1; 3) 264–58; a duplication of a small euchromatic section of X (3B 3·4 to 3D 5·6 inclusive) which is inserted in the chromocenter of 3L proximal to 80C (Plate 1, A).

* The cost of the accompanying plates has been borne by the Galton and Mendel Memorial Fund.

2) T(1; 2; 4) 264–85, a complex translocation in which a euchromatic section of X (3C1 to 6A 1·2 inclusive) is inserted in the chromocenter of 4 between 101F and 102A. (Plate 1, B).

3) T(1; 4) 264–86, a translocation in which a piece of X (3C7 to 3E5 inclusive) is inserted in the chromocenter of 4 at 101F (Plate 1, C).

4) T(1; 3) 264–100, insertion of a piece of X (3C1 to 4B 3·4 inclusive) in the chromocenter of 3 between 80C and 81F (unmapped region). (Plate 1, E).

B. Translocations of 3C bands to heterochromatic regions.

5) T(1; 3) 264–70, a translocation in which heterochromatin of 3L is adjacent to 3C4 and 3C 5·6 (Plate 1, D; Plate 2, G).

6) T(1; 2; 3) 264–74, a complex translocation in which 3C 9·10 is adjacent to heterochromatin of 2L. (Plate 1, F).

C. Insertions of 3C bands into euchromatic regions.

7) Tp(1) 264–63, a transposition in which 3C 9·10 and the succeeding bands are adjacent to 13B.

8) T(1; 2; 3) 264–87, a complex translocation with a piece of X (3C 9·10 to 10A 1·2 inclusive) inserted in 2R between euchromatic segments 60A and 45E.

9) T(1; 3) 258–44, a complex translocation with a piece of X (3C4 to 4D 1·2 inclusive) inserted in 2R between 56 E1 and F1.

D. Insertions of other regions into chromocenter.

10) T(1; 3) 268–37, insertion of a piece of X (5D 3·4 to 7B 1·2 inclusive) in 3L chromocenter proximal to 80C. (Plate 2, H).

11) T(1; 2; 3) 268–40 a complex translocation with a piece of 3R (87D3 to 88C 2 inclusive) inserted in the chromocenter of 2L between 40F and 41A (Plate 2, J).

E. Insertion of heterochromatin into euchromatic region.

12) T(1; 2) 258–36, a complex translocation in which a heterochromatic section of 2R (41B to 41F) is inserted between two euchromatic regions of X, following 3C 5·6 and preceding 4C 3·4 (Plate 2, I).

F. Rearrangement involving 2B region and chromocenter.

13) In (1) 313–25–36. Inversion of a section of X from 2B ("bulb" region) to the chromocenter. (Plate 2, K).

METHODS

Slides of the salivary gland chromosomes of female larvae heterozygous for the rearrangements concerned were prepared by both aceto-carmine and Feulgen techniques. The larvae were raised in the cold room at a constant temperature of 18°–19°C.

For analysis of the slides, the equipment consisted of a 90×, 1.3 N. A. apochromatic objective, an oil-immersed 1.4 N. A. condenser, 12.5×

compensating oculars, and a Bausch & Lomb research lamp with the green Wratton filter number 61 which transmits light waves between 4500 and 6100 A° and has the maximum at about 5200 A°.

Drawings were made with the aid of a camera lucida at an initial magnification of c. 3000×.

Cytological analyses were made with the aid of BRIDGES' (1935; 1938) salivary chromosome maps.

OBSERVATIONS AND CONCLUSIONS

It must be admitted that observations on changes such as these are difficult, because the chromocenter region is very easily broken up by the pressure used in making slides, and the loosely organized bands may be smeared over adjacent chromosome sections so as to obscure the euchromatic bands. Figure F on Plate 1 shows an unquestionable example of this. I have interpreted several figures in this way, though they might conceivably be interpreted as being deficient in one or more euchromatic bands immediately adjacent to the chromocenter.

In several of the rearrangements (numbers 1–6 described above) the euchromatic bands involved were those of the white-Notch region (section 3C of the X chromosome). These bands are very well defined and easily recognizable (Figure 1 second from top). In each case a break had been induced in this region, and one or both of the broken ends had become attached to a heterochromatic region where a second break had occurred. The change was associated with mottling of one or more characters determined by genes in the white-Notch region.

At the temperature at which the larvae were raised (18° to 19°C), mottling was extreme (most of the tissue showing the mutant character, with a few patches of wild-type tissue). The slides were examined carefully for the presence of extra Y chromosomes which, according to SCHULTZ, increase the amount of wild-type tissue in phenotypic characters and reduce the variation in the appearance of the salivary bands. No extra Y chromosomes were found in the salivary glands used in this study.

In Dp(1) 264–58, where twenty euchromatic bands of X were inserted in the 3L chromocenter, figures were seen in which all the bands were readily identifiable and the appearance of these bands in their new position was strictly comparable with that in their normal position (Plate 1, A). The same was true in T(1; 2; 4) 264–85, where forty bands of X, beginning

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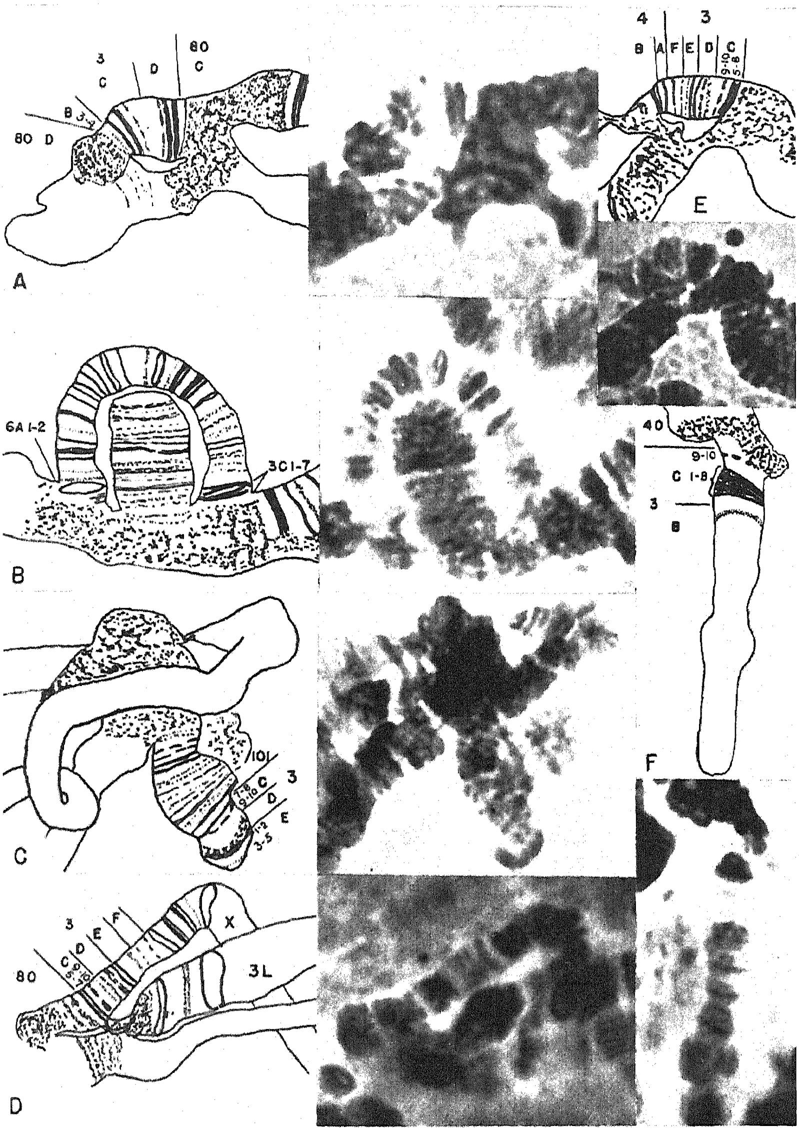
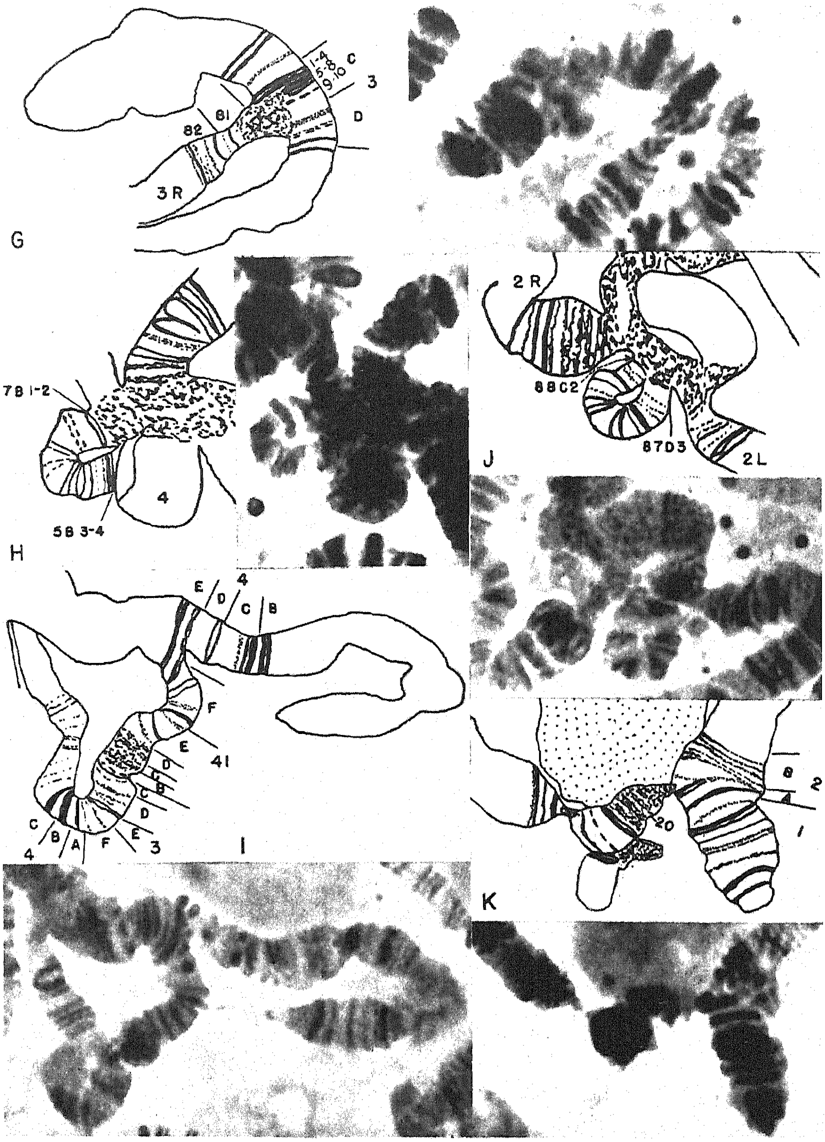
EXPLANATION OF PLATE 1

Photomicrographs and diagrams of Drosophila salivary chromosomes with following chromatin exchanges, A. Dp(1;3) 264.58; B. T(1;2;4) 264.85; C. T(1;4) 264.86; D. T(1;3) 264–70; E. T(1;3) 264.100; F. T(1;2;3) 264.74. For further description, see text.


A
B
C
D
E
F
6A 1-2
3C 1-7
3L
X
40
80


G
3R
81
82
C
3
D
H
78 1-2
58 3-4
4
J
2R
88C2
87D3
2L
I
41
3
K
20
2
1

at the 3C region, were inserted in the chromocenter of the fourth chromosome (Plate, 1, B); and in T(1; 4) 264–86 and T(1; 3) 264–100, which showed similar insertions of different lengths (Plate 1, C and E).

Occasionally a band near to a heterochromatic region appears darker than the corresponding band in the normal chromosome; but in other cells the same band may be lighter than normal. In T(1; 3) 264–70 part of the 3L chromocenter was attached to X before 3C 5·6 (Plate 1, D) and though the 3C bands were unaffected, the next group of dark bands in X (3D–E) following the heterochromatic (80D) region of 3L appeared darker than normal in some cells. A count was made to determine the proportion of such occurrences, with the following results:—

3D–E similar to normal—33 figures
3D–E darker than normal—5 figures
3D–E lighter than normal—1 figure

It seems probable that the variation in intensity, occurring in both directions, is due to random variation in the distribution of stain rather than to a specific effect of the neighboring heterochromatin.

As a control, a study was made of Tp(1) 264–63, T(1; 2; 3) 264–87, and T(1; 3) 258–44, in all of which sections of X including the 3C bands were inserted between euchromatic bands. In some figures homologous bands in the normal and reconstituted chromosomes showed with a different intensity.

In two of the translocations described above (Dp (1; 3) 264–58 and T(1; 4) 264–86) the limiting euchromatic bands at one end of the piece inserted in heterochromatin were light bands (Plate 1, A and C), while in all other cases the euchromatic band nearest to the heterochromatin was a heavy band. It was found that the light band 3B 3–4 in Dp(1; 3) 264–58 and the light bands 3F 3–5 in T(1; 4) 264–86 were not always visible; but this cannot be considered remarkable, as even in their normal position these bands are distinctly seen only in the best preparations. Wherever the euchromatic band nearest to the heterochromatin was heavy, this band was nearly always visible, and was indistinguishable only when obscured by spreading of the chromocenter.

Two other rearrangements showed insertions of other regions than 3C into the chromocenter. In one of these (T(1; 3) 268–37) an insertion of a piece of X was limited on both sides by heavy bands which could always be clearly seen (Plate 2, H). In the other (T(1; 2; 3) 268–40) a piece of 3R

EXPLANATION OF PLATE 2

Photomicrographs and diagrams of Drosophila salivary chromosomes with following chromatin exchanges, G. T(1;3) 264.70; H. T(1;3) 268.37; I. T(1;2) 258.36; J. T(1;2;3) 268.40; K. In(1) 313.25.36. For further description see text.

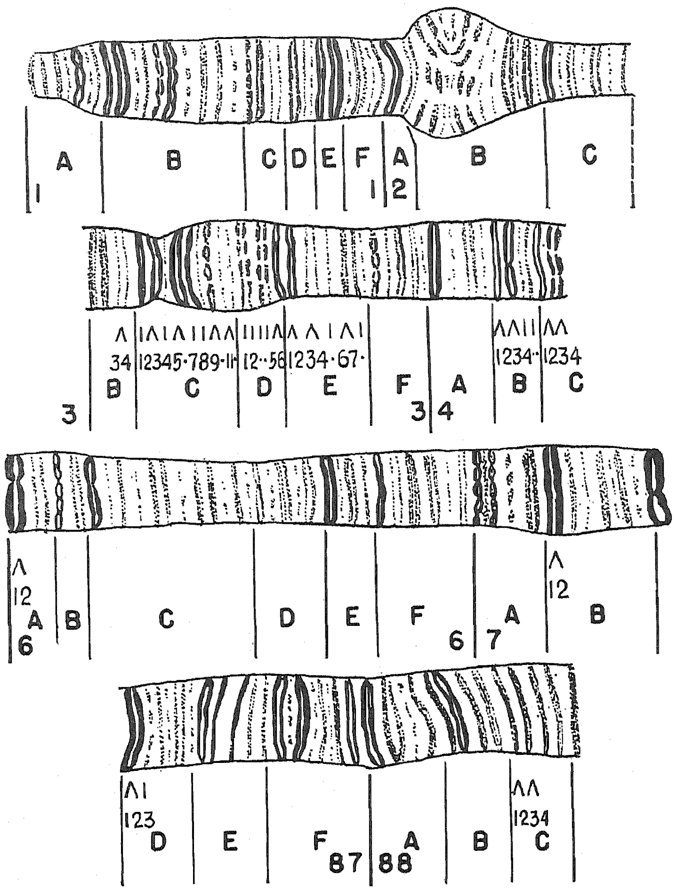


FIGURE 1.—Diagrams from Bridges' salivary chromosome maps showing three regions of X and (bottom) one of 3R, involved in translocations to heterochromatin; for comparison with drawings and photographs in Plates 1 and 2.

(figure 1 bottom) was inserted in the 2L chromocenter, and was limited at one end by the light bands 87D 3–8, at the other by 88C 2, a band of medium intensity. The six light bands in 87D cannot always be distinguished individually, but all figures showed that some, if not all, of the group were present (Plate 2, J), while 88C 2 was clearly visible.

These rearrangements provided no evidence for an effect of heterochromatin on the neighboring euchromatic bands, apart from the disturbed figures whose interpretation has been discussed above.

The converse effect of euchromatic regions on heterochromatic insertions was not apparent in this material. In T(1; 2) 258–36 part of the 2R chromocenter was inserted in X between the 3C 5–6 and 4C 1–2 bands, and the structure of the heterochromatic bands remained the same as it is when they are in the normal position (Plate 2, I). T(1; 3) 264–70 and T(1; 2; 3)

264–74, in which heterochromatic regions of other chromosomes were translocated to the 3C region, showed no change in the heterochromatin (Plate 2, G).

Evidence of change was found, however, in one rearrangement provided by DR. B. P. KAUFMANN. This was an inversion in the X chromosome in which the left break was in the 2B ("bulb") region (see figure 1, top) and the right break was in the chromocenter. The 2B bands were reattached in an inverted position to the chromocenter, and they frequently showed darker, but never lighter, than in the normal strand. Only one slide was available as it came from an experiment in which F_1 larvae from irradiated males were used for slides; in eighteen clear figures the bands in both strands of X appeared to be of like intensity, while in ten figures the 2B bands adjacent to heterochromatin were darker than those in the normal position. (Plate 2, K). No loss of bands was apparent in any of the figures.

According to PROKOFYEVA-BELGOVSKAYA (1938), the "bulb" region of X is peculiar in that it consists of inert material homologous with the chromocenter; and if this is so, the exceptional case just described cannot be considered as an example of the influence of heterochromatin on euchromatic bands.

It does not seem necessary to assume that the darkening of the bands is caused by the proximity of heterochromatin. If it were, the 3C region, which also is supposed by PROKOFYEVA-BELGOVSKAYA (1939) to consist of heterochromatic material, should be changed when transferred to the chromocenter; but as the bands of this region remain unchanged, the effectiveness of heterochromatin in producing such changes is open to question.

Whether the 3C region be considered as heterochromatic and comparable to 2B or as a series of euchromatic bands with which known loci are associated, the constancy of these bands tends to refute the suggestion of a heterochromatin effect. The evidence presented here as to the behavior of other euchromatic regions casts further doubt on the existence of such an effect.

As the observational methods used for this work are liable to subjective errors, it is possible that the photometric methods of CASPERSSON and SCHULTZ (1939) would give different results; and a further study of the material is being made by the latter methods.

SUMMARY

In a study of euchromatic bands transferred to heterochromatic regions, and vice versa, evidence of change in structure was found in a single case, where bands from 2B transposed to the chromocenter of X sometimes appear darker than the homologous bands in the normal X. Bands from the

white-Notch region of X (3C) do not appear to be lost or changed when they are adjacent to heterochromatin, nor do bands from some other euchromatic regions of the chromosomes.

Heterochromatic regions inserted between euchromatic bands retain their characteristic structure.

It is concluded that these studies provide no evidence of visible change or loss of bands due to an interaction between euchromatin and heterochromatin.

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POSITION EFFECT AND GENE DIVISIBILITY CONSIDERED IN CONNECTION WITH THREE STRIKINGLY SIMILAR SCUTE MUTATIONS[1]

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DANIEL RAFFEL AND H. J. MULLER
Baltimore, Maryland, and The University, Edinburgh, Scotland

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BACKGROUND OF THE PRESENT WORK

AFTER the proposal of the subgene theory by SEREBROVSKY, DUBININ and their co-workers (SEREBROVSKY and DUBININ, 1929 *et seq.*), in interpretation of the relationships observed by them among scute alleles, an attempt was made by MULLER and co-workers, first at the University of Texas laboratory and later at the Institute of Genetics of the U.S.S.R., to obtain further scute alleles and to analyse these and the ones previously obtained elsewhere, with a view to further testing the above theory and to further investigation of the curious relationship which he had observed between "gene mutations" and "gene rearrangements" (MULLER 1930a, b, 1932). It was soon evident that a high proportion of the scute mutations involved the breakage and reattachment of the X chromosome very close to the locus of scute, but it was not clear whether the phenotypic change represented a "gene mutation" that was near to but, theoretically at least, separable from the rearrangement, or whether it represented the "position effect" of the gene rearrangement itself.

We shall not describe here the work of analysing the position of breakage of these rearrangements of the scute region with respect to each other, reported by MULLER and PROKOFYEVA (1934, 1935a) and briefly mentioned below (p. 566). Suffice it here to say that the numerous rearrangements, although all appearing to possess one point of breakage and interchange of gene connections in one of four definite positions near scute had their other point of breakage anywhere in the X or other chromosomes, so that the gene arrangements occurring in the neighborhood of scute, after the breakages and reattachments had taken place, were in all cases very different from those in the normal chromosome and (with the exceptions to be noted presently) from each other. Correspondingly there was very much diversity in the phenotypic expression of the 'alleles,' although no fixed relation was discernible between the map position of the different

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chromosome breaks and the kinds of scute phenotype associated with them.

Evidence was, however, obtained that a strong relationship did exist between the type of rearrangement and the type of phenotypic change when two scute mutations that had not previously been studied were subjected to analysis. These were "scute-L8," found by H. LEVY in Texas in 1932, and "scute-S1," found by T. G. SINITSKAYA in Leningrad in 1934. It was found in genetic investigations of the senior author that these two mutants had their X chromosomes broken at sensibly the same place in the scute region, and had them broken also in the right-hand chromocentral ("inert") region of the X, the part of the chromosome between these two breaks being inverted with respect to the rest. In all these particulars, moreover, these two rearrangements agreed with the already known inversion found in the mutant scute-4. (Tests made later did, however, show some difference in the position of the breaks in the chromocentral region, that in scute-4 being to the left of bobbed and block A, that in scute-L8 between them, and that in scute-S1 to the right of both.) By themselves, these coincidences would not be so very striking, since, in the first place, scute mutations (already known often to be associated with breaks near the scute locus) were being searched for, and since, secondly, breaks in the chromocentral region occur relatively frequently. What made the findings striking was the fact that, to parallel this unusual resemblance in type of gene rearrangement, there was also a similar strong resemblance between the types of phenotypic expression of all three of them. In fact, the phenotypic expressions of all three were more like one another than any of them was like any of the numerous other scute mutations known, just as the gene arrangements were more like one another than any of them was like that of any other scute.

These facts were first known only for scute-L8 and scute-4, and were taken by MULLER and PROKOFYEVA (1934, 1935a) as furnishing, along with other facts, strong evidence for their conclusion that the phenotypic changes caused by the so-called mutations accompanying chromosomal rearrangements are in general, except in so far as they are due to losses or duplications, manifestations of a "position effect" on the genes lying near the points of breakage. The later results on scute-S1, agreeing with those on scutes-4 and -L8, seemed to remove all possibility of the agreement between gene arrangement and phenotype being a matter of coincidence.

In view of the significance of such a relationship for our understanding of mutations, both spontaneous and induced, and in order to learn something more concerning the nature of the so-called position effect, it was decided to make a more accurate study of the phenotypic effects of these

three scute mutations, in stocks that were as nearly isogenic as possible. For, despite the phenotypic similarity of these three mutations, certain differences had been observed between them, as well as peculiarities of the recombinational forms, as for example the sterility of crossover males with the left part of the scute-4 and the right part of the scute-L8 chromosomes. These had not been subjected to quantitative study in the earlier work because it was not yet known to what extent they might be due to differences in environmental conditions or in other genes (modifying factors that might lie anywhere in the X chromosome or the autosomes), and to what extent the differences were localisable in and near the gene for scute itself and the right-hand points of breakage.

The expression of scute is known to be rather sensitive to differences in environic factors and modifying genes, and there was no reason to regard the original stocks of the three inversions as containing fewer "invisible" differences in their genes—either in their X chromosomes or autosomes—than most stocks do. In fact, scute-S1 was known to carry a smaller inversion, morphologically somewhat similar to the known inversion called delta 49, in its X chromosome, entirely included within the larger inversion. This same stock of scute-S1 was also known to have an inherited tendency to nicking at the ends of the wings. All this made it the more important to make a comparison on groups of flies which had been made isogenic and in which the effects of environmental differences were minimized. The present paper gives a report of this study on these three scutes. In addition, the effects were studied of the various possible combinations having the left part of one of the inversions and the right part of another, in stocks similarly isogenic.

The results of this investigation show that the phenotypic expressions of these three mutations are, even when subjected to this more exact comparison, very similar to one another but that they are not identical. It is found that both the left and right regions containing the points at which the breaks and recombinations occurred (hereafter referred to as the left and right "ends" of the inversions) appear to affect the development of the bristles. However, while the influences of the left regions (including the "locus of scute" itself) are unquestionable and regular, analysis of the right regions shows them to be much weaker, more uncertain and inconsistent, and it is possible that they do not represent any real effect of the right ends themselves.

The above findings raise important questions of interpretation, concerning the position effect, gene divisibility, etc., which are herein discussed in the light of theoretical considerations and of related work on these subjects.

MATERIALS AND METHODS

In order to obtain differences in the expression of the scute mutations in as nearly as possible identical genetic associations, the chromosomes containing the three original inversions were subjected to crossing so as to replace the whole central portion of the chromosomes, extending from some point between the left point of breakage and the locus of white to a point between the locus of carnation and the right point of breakage (more than 80 percent of the length of the chromosome as seen in the salivary gland cells), by the corresponding region derived from a given X chromosome containing certain mutant genes (apricot, w^a, locus 1.5, miniature, *m*, 36.1, and carnation, *car*, 62.5) that served as markers. The obtaining of such isogenic X chromosomes was especially difficult in the case of scute-S1 because of the initial presence in this chromosome of the smaller inversion, included within the larger one, which greatly reduced crossing over on both sides of it and especially to the right of it. The reconstructed and as nearly as possible isogenic X chromosomes were then introduced into a stock that was isogenic for all the genes in the major portions of the two long autosomes, and thus males were obtained that were isogenic for all genes except those in the small chromosome, IV, and near the very ends of the other chromosomes.

The isogenic X chromosomes were obtained by the series of crosses shown below. In the notation used here the females are represented first except where otherwise stated. The crosses of generations that follow one another are designated by successive numbers, and letters are added to distinguish different lines of descent. Individuals deriving from any given cross either immediately or, in the case of the various stocks made in the course of this work, through a succession of crosses of the same kind, are designated by an "F" in parenthesis with the number of that cross or stock as a subscript. The notations L and R are used to denote respectively the left and right ends of the inversions that had been associated with the mutations designated. These letters are used only in cases where, as a result of crossing over, the two ends of a given inversion are derived from different original inversions.

1. *y sc-4 B*$\times$*car*
2. (F_1)*y sc-4 B/car*$\times$*y sc-4 B*
3. *sc-8 m* $w^a$$\times$$(F_2)$*y sc-4 car*
4. (F_3)*sc-8 m* w^a*/y sc-4 car*$\times$*sc-8 m* w^a
5. $\widehat{yy}$$\times$a single (F_4)*y sc-4L car m* w^a *sc-8R* (stock)

6a. *sc-L8/w* lz^s dl 49$\times$$(F_5)$ *y sc-4L car m* w^a *sc-8R*

7a. (F_{6a})*sc-L8/y sc-4L car m* w^a *sc-8R*$\times$*y sc-13 w*

8a. (F_{7a})*y sc-4L car m* w^a *sc-L8R/y sc-13 w*$\times$*y w* lz^s dl 49

9a. (F$_{8a}$)y sc-4L car m w^a sc-L8R/y w lz^s dl 49 × y w lz^s dl 49 (stock)
8b. $\widehat{yy}$ × (F$_{7a}$)sc-L8L car m w^a sc-8R (stock)
9b. y sc-4 × (F$_{8b}$)sc-L8L car m w^a sc-8R
10b. (F$_{9b}$)y sc-4/sc-L8L car m w^a sc-8R × y sc-13 w
11b. (F$_{10b}$)sc-L8L car m w^a sc-4R/y sc-13 w × y w lz^s dl 49
12b. (F$_{11b}$)sc-L8L car m w^a sc-4R/y w lz^s dl 49 × y w lz^s dl 49 (stock)
13b. (F$_{12b}$)sc-L8L car m w^a sc-4R/y w lz^s dl 49 × (F$_5$)y sc-4L car m w^a sc-8R
14b. (F$_{13b}$)sc-L8L car m w^a sc-4R/y sc-4L car m w^a sc-8R × y sc-13 w
15b. (F$_{14b}$)y sc-4 car m w^a/y sc-13 w × y w lz^s dl 49
16b. (F$_{15b}$)y sc-4 car m w^a/y w lz^s dl 49 × y w lz^s dl 49 (stock)
10c. (F$_{9a}$)y sc-4L car m w^a sc-L8R/y w lz^s dl 49 × (F$_{8b}$)sc-L8L car m w^a sc-8R
11c. (F$_{10c}$)y sc-4L car m w^a sc-L8R/sc-L8L car m w^a sc-8R × y sc-13 w
12c. (F$_{11c}$)sc-L8 car m w^a/y sc-13 w × y w lz^s dl 49
13c. (F$_{12c}$)sc-L8 car m w^a/y w lz^s dl 49 × y w lz^s dl 49 (stock)
6d. sc-S1/w lz^s dl 49 × (F$_5$)y sc-4L car m w^a sc-8R
7d. (F$_{6d}$)sc-S1/y sc-4L car m w^a sc-8R × y sc-13 w
8d. (F$_{7d}$)sc-S1L car m w^a sc-8R male × $\widehat{yy}$ (stock)
9d. (F$_{9a}$)y sc-4L car m w^a sc-L8R/y w lz^s dl 49 × (F$_{8d}$)sc-S1L car m w^a sc-8R
10d. (F$_{9d}$)y sc-4L car m w^a sc-L8R/sc-S1L car m w^a sc-8R × y sc-13 w
11d. (F$_{10d}$)sc-S1L car m w^a sc-L8R/y sc-13 w × y w lz^s dl 49
12d. (F$_{11d}$)sc-S1L car m w^a sc-L8R/y w lz^s dl 49 × y w lz^s dl 49 (stock)
17a. (F$_{16b}$)y sc-4 car m w^a/y w lz^s dl 49 × (F$_{8d}$)sc-S1L car m w^a sc-8R
18a. (F$_{17a}$)y sc-4 car m w^a/sc-S1L car m w^a sc-8R × y sc-13 w
19a. (F$_{18a}$)sc-S1L car m w^a sc-4R/y sc-13 w × y w lz^s dl 49
20a. (F$_{19a}$)sc-S1L car m w^a sc-4R/y w lz^s dl 49 × y w lz^s dl 49 (stock)
13e. (F$_{12b}$)sc-L8L car m w^a sc-4R/y w lz^s dl 49 × (F$_{7d}$)y sc-4L car m sc-S1R
14e. (F$_{13e}$)y sc-4L car m sc-S1R/y w lz^s dl 49 × y w lz^s dl 49 (stock)
15e. (F$_{14e}$)y sc-4L car m sc-S1R/y w lz^s dl 49 × (F$_{8d}$) sc-S1L car m w^a sc-8R
16e. (F$_{15e}$)y sc-4L car m sc-S1R/sc-S1L car m w^a sc-8R × y sc-13 w
17e. (F$_{16e}$)sc-S1 car m w^a/y sc-13 w × y w lz^s dl 49
18e. (F$_{17e}$)sc-S1 car m w^a/y w lz^s dl 49 × y w lz^s dl 49 (stock)
19e. (F$_{18e}$)sc-S1 car m w^a/y w lz^s dl 49 × (F$_5$)y sc-4L car m w^a sc-8R
20e. (F$_{19e}$)sc-S1 car m w^a/y sc-4L car m w^a sc-8R × y sc-13 w
21e. (F$_{20e}$)y sc-4L car m w^a sc-S1R/y sc-13 w × y w lz^s dl 49
22e. (F$_{21e}$)y sc-4L car m w^a sc-S1R/y w lz^s dl 49 × y w lz^s dl 49 (stock)
23e. (F$_{22e}$)y sc-4L car m w^a sc-S1R/y w lz^s dl 49 × (F$_{8b}$)sc-L8L car m w^a sc-8R
24e. (F$_{23e}$)y sc-4L car m w^a sc-S1R/sc-L8L car m w^a sc-8R × y sc-13 w
25e. (F$_{24e}$)sc-L8L car m w^a sc-S1R/y sc-13 w × y w lz^s dl 49
26e. (F$_{25e}$)sc-L8L car m w^a sc-S1R/y w lz^s ld 49 × y w lz^s dl 49 (stock)

Having thus made up stocks of the three inversions and of all six possible recombinations of their ends, so constituted that the whole central region of each of these nine X chromosomes between the loci of white and carnation, inclusive, was the direct descendant of the same original *y sc-4L car m* w^a *sc-8R* chromosome, it was necessary to introduce each of these nine X chromosomes into a stock that had been made isogenic for the two long autosomes. This stock had been prepared by the series of crosses shown below. In what follows "II" and "III" represent those normal second and third chromosomes which entered into the composition of the isogenic stock. The presence of other normal chromosomes is indicated only by blank spaces in the appropriate positions.

1f. *Cy sp/apl*×*ru h D* CXF *ca/rucuca*
2f. (F_{1f})*Cy sp/*; *ru h D* CXF *ca/*×*apl/*; *rucuca/*
3f. (F_{2f})*Cy sp/apl*; *ru h D* CXF *ca/rucuca* (stock)
4f. $\widehat{yy}$; II/; III/×(F_{3f})*Cy sp/apl*; *ru h D* CXF *ca/rucuca*
5f. (F_{4f}) single female $\widehat{yy}$; *Cy sp*/II; *ru h D* CXF *ca*/III
  ×(F_{3f})*Cy sp/apl*; *ru h D* CXF *ca/rucuca*
6f. (F_{5f})$\widehat{yy}$; *Cy sp*/II; *ru h D* CXF *ca*/III
  ×(F_{5f})*Cy sp*/II; *ru h D* CXF *ca*/III
7f. (F_{6f})$\widehat{yy}$; II; III×II; III (stock, called below the "M" stock)

Here *CXF* represents a combination of inversions in the third chromosome effectively preventing all crossing over except a small amount near the ends. It lies in the same chromosome with the dominant marker "Dichaete" (*D*). The obtaining of this useful combination involved a series of steps carried out with the intention of obtaining such a result. After preliminary radiations of Dichaete by MULLER, which had yielded only some inversions permitting considerable crossing over, a much better combination, called CX, was obtained by OLIVER in Texas in 1929; and further irradiation of the latter chromosome, first by STONE in Texas and then by FYODOROVA in Leningrad in 1935 (see D.I.S., 6), resulted in the present CXF combination.

"*rucuca*" represents a well-known combination of third chromosome mutant genes consisting of: roughoid (*ru*), 0.0; hairy (*h*), 26.5; thread (*th*), 42.2; scarlet (*st*) 44.0; curled (*cu*), 50.0; stripe (*sr*), 62.0; sooty (e^s), 70.7; and claret (*ca*), 100.7.

"*Cy*" represents Curly, which is a spontaneous dominant mutation in the second chromosome associated with two inversions, one suppressing crossing over throughout the major portion of the left arm of the second chromosome and the other throughout the major part of the right arm.

"*apl*" represents a well-known combination of second chromosome mutant genes consisting of: aristaless (*al*), 0.0; dumpy (*dp*), 13.0; black

(*b*), 48.5; purple (*pr*), 54.5; curved (*c*), 75.5; plexus (*pl*), 100.5; and speck (*sp*), 107.0.

The stock, "M," obtained in the cross "7f" was homozygous for all the genes in the long second and third chromosomes except possibly those near the very ends. The nine scute-containing X chromosomes isogenic for all but their end regions were now brought into association with the second and third chromosomes of stock "M" by means of a series of crosses, all of the type defined below. There were nine such series, begun with females derived from F9a, F12b, F12d, F13c, F16b, F18e, F20a, F22e, and F26e, respectively. In what follows, the nine X chromosomes from these stocks, containing any of the three original scute mutations or the six recombinations of the ends of the inversions associated with them, are designated simply as "scute."

27. "scute"/*y w* lz^s dl 49 × (F_{3f})*Cy sp*/*apl*; *ru h D* CXF *ca*/*rucuca*
28. (F_{27})"scute"/; *Cy sp*/; *ru h D* CXF *ca*/ × (F_{7f})II; III (stock "M")

By breeding together flies from the above cross (28), heterozygous for the long autosomes of the "M" stock (F_{7f}) and the crossover-suppressing chromosomes marked by *Cy* and *D*, (that is, flies showing Curly and Dichaete but not roughoid, hairy, claret, or speck), males with the desired X chromosomes and homozygous for the second and third chromosomes of the "M" stock were produced. It was found, however, that such males were too inviable to appear in sufficient numbers for the purpose of this investigation. To obviate this difficulty females obtained from cross (28) were mated with males from the homozygous stock from Amherst which PROFESSOR PLOUGH kindly furnished us with and which is referred to below as stock "A." The final cross involving each of the nine isogenic lines then was

29. (F_{28}) "scute"/; *Cy sp*/IIM; *ru h D* CXF *ca*/IIIM × the normal stock "A."

These crosses yielded males (F_{29}) "scute"; IIM/IIA; IIIM/IIIA.

To recapitulate, then, males of this kind were obtained with each of the nine kinds of X chromosomes marked with w^a, *m*, and *car*, to ensure that the X chromosomes, at least between the loci of *w* and *car*, as well as the two long autosomes, except for their very ends, would be the same in all of the flies of all nine types of scute combinations.

In order to minimize the differences in cultural conditions, the crosses yielding all these nine final types (F_{29}) were made simultaneously, in vials containing food derived from the same lot, and each of these nine crosses was at the same time made in replicate, in a series of ten or more vials. The bristles on both sides of approximately 25 males of each of the nine

types, derived in approximately equal proportions from all the ten cultures of a given type, were recorded (thus giving approximately fifty sets of bristles in each case).

RESULTS

Classes of effects on the bristles

In table 1 we present the average number of bristles of each kind for the nine types of F_{29} males studied, together with the standard errors of these averages as calculated by dividing the standard deviations found by $\sqrt{n}$, n being the number of flies on which the average was based. The first three columns give the results for the three original inversions, scute-4, scute-S1, and scute-L8, and the last six columns give the results for the various possible recombinational types. An inspection of these columns will show that although these three scute mutations and their combinations are strikingly similar to one another and indeed much more so than to any other scute mutations hitherto described, yet no two of the nine types seem to have exactly the same phenotypic expression and, so far as the three original types are concerned, wherever significant differences in the frequency of given bristles occur the order of frequency (from higher to lower bristle number) is always scute-4, scute-S1, scute-L8.

Several bristles were not significantly changed from the normal number by any of these three inversions or recombinations of them, or the change was so slight that the values of all the nine groups might be regarded as virtually random deviations from the same nearly-normal figure. Among these bristles were the internal verticals, the posterior dorsocentrals (except for some slight fluctuations), and the posterior sternopleurals. The same applies to the thoracic microchaetes. All of these except perhaps the posterior sternopleurals belong to the "achaete" group; that is, they do not ordinarily seem to be absent as a result of loss or change of the scute locus, but rather of the neighboring locus of achaete.

Other bristles were entirely absent in all nine types of scutes, or were absent in some types and appeared in the others in such very small numbers that the differences between the frequencies observed in any of the types had little or no statistical significance. The bristles so greatly reduced as this were the post-verticals, the external verticals, the internal postalars, the two humerals, the two scutellars, the coxals, the ventrals, the first and third orbitals, and the external supraalars. The last, however, in just one group, that of scute-S1, had a bristle frequency such that a deviation nearly as great as twice its own standard error would have been required to give a value of zero. There are three other bristles that belong in nearly the same class as the preceding, except that each of them deviates more widely from the zero frequency than allowed on the above

scheme in just one of the nine groups of flies. These bristles are the second orbitals (deviating in scute-4L·scute-L8R), the presuturals (also deviating in scute-4L·scute-L8R) and the anterior notopleurals (deviating in scute-4L·scute-S1R).

There is another group of bristles—the ocellars, the sternopleural microchaetes and the middle sternopleurals—which are partially reduced in frequency but, unlike most bristles that are partially reduced, are affected to sensibly the same degree in all nine groups—or, in the case of the middle sternopleurals in all but one of the nine groups, with the one exceptional case representing only a slight deviation from the rest.

Except for the isolated and not very marked cases of deviation above mentioned—which can hardly be used for the drawing of definite conclusions regarding the effects of left and right ends—the significant differences in bristle frequencies are largely confined to the other bristles, and we are therefore grouping these more significant bristles together in the tables so as to facilitate comparison. These bristles include the following: the anterior dorsocentrals and the internal supraalars (these as well as the internal verticals, posterior dorsocentrals, and thoracic microchaetes mentioned above are the chaetae which are especially affected by changes involving the locus of achaete, to the left of that of scute); the external postalars, the posterior notopleurals, the anterior sternopleurals, the dorsal abdominal microchaetes and macrochaetes and the three sets of sternitals. The latter we may group together for consideration, as the variations of all three sets of sternitals proved to be consistently parallel. For the reasons above given, we shall in what follows confine our attention to the bristles of these eight types—or ten types, when we take the three types of sternitals separately. The values found for them are given in the first ten rows of the tables.

Comparison of effects of the original inversions

Considering the frequencies of the latter bristles only in the first three columns of table 1 we see that all three of these original inversions, although on the whole strikingly similar to one another and indeed much more so than to any other scute mutation previously described, have their own distinctive frequencies for these particular bristles in the great majority of cases. The so-called achaete bristles mentioned above (anterior dorsocentrals and internal supraalars) were not affected by the scute-4 inversion, were barely significantly reduced by the scute-S1 inversion and were reduced nearly to one half their normal frequency by the scute-L8 inversion. The extent of reduction of the external postalars, like that of the achaete bristles, was, in order of increasing reduction: scute-4, scute-S1, scute-L8, the reduction in the step from the first to the

TABLE I

Bristle frequencies for each scute combination.

CLASSES OF BRISTLES	BRISTLES	SC-4	SC-S1	SC-L8	SC-4L · SC-S1R	SC-4L · SC-L8R	SC-S1L · SC-4R	SC-S1L · SC-L8R	SC-L8L · SC-4R	SC-L8L · SC-S1R
1	dorsocentrals (ant.)	2.00±0.00	1.86±0.06	1.39±0.13	2.00±0.00	1.96±0.04	2.00±0.00	1.91±0.06	1.70±0.11	1.52±0.09
	supraalars (int.)	2.00±0.00	1.86±0.06	1.46±0.09	2.00±0.00	1.93±0.05	1.81±0.10	1.86±0.07	1.70±0.10	1.62±0.07
	postalars (ext.)	1.61±0.12	1.34±0.11	1.14±0.11	1.88±0.16	2.00±0.00	1.56±0.12	1.55±0.18	1.07±0.15	1.16±0.10
	notopleurals (post.)	1.89±0.06	0.80±0.14	0.68±0.13	2.00±0.00	2.00±0.00	1.31±0.17	1.36±0.15	0.37±0.10	0.56±0.09
	sternopleurals (ant.)	2.00±0.00	1.34±0.13	1.07±0.15	2.00±0.00	1.71±0.08	1.25±0.14	1.73±0.10	0.60±0.15	0.98±0.11
	sternitals (seg. 2)	5.14±0.15	2.83±0.21	1.48±0.22	4.70±0.15	4.50±0.16	2.75±0 29	2.23±0.24	2.33±0.21	2.18±0.19
	sternitals (seg. 3)	5.18±0.29	3.03±0.19	1.32±0.19	4.76±0.16	4.93±0.17	2.50±0.25	2.09±0.23	2.50±0.19	2.26±0.17
	sternitals (seg. 4)	5.47±0.16	2.89±0.25	1.93±0.23	4.87±0.20	4.72±0.16	3.25±0.24	2.41±0.23	2.83±0.19	2.18±0.16
	dorsal abd. micro.	+	=	≡	–	–	=	≡	=	=
	dorsal abd. macro.	–	≡	o	–	=	≡	o+	≡	o+
2	ocellars	1.86±0.06	1.71±0.10	1.68±0.10	1.71±0.16	1.29±0.15	1.69±0.15	1.73±0.12	1.70±0.11	1.50±0.33
	sternopleurals (macro)	0.14±0.07	0.11±0.05	0.18±0.09	0.08±0.09	0.18±0.09	0.25±0.14	0.32±0.13	:	0.16±0.06
	sternopleurals (micro)	10.36±0.30	10.63±0.24	10.47±0.31	10.78±0.32	10.39±0.19	10.63±0.35	10.86±0.75	9.83±0.20	10.40±0.20
3	orbitals (1st)	:*	0.03±0.03	:	0.12±0.08	0.07±0.05	:	0.05±0.04	:	0.04±0.03
	orbitals (2nd)	0.11±0.08	0.09±0.05	0.14±0.06	0.18±0.09	0.89±0.15	0.13±0.09	0.05±0.04	:	0.02±0.02
	orbitals (3rd)	:	0.09±0.05	:	:	0.04±0.04	0.06±0.06	:	:	
	postverticals	:	0.03±0.03	:	:	0.04±0.04	:	0.09±0.06	:	:
	verticals (ext.)	:	:	:	:	:	:	:	:	:
	supraalars (ext.)	:	0.09±0.05	:	:	:	:	:	:	:
	postalars (int.)	:	:	:	:	:	:	:	:	:
	presuturals	:	0.09±0.05	:	0.06±0.06	0.21±0.09	:	.09±0.06	:	:
	humerals (dors.)	:	:	:	:	:	:	:	:	:
	humerals (vent.)	:	:	:	:	:	:	:	:	:
	notopleurals (ant.)	0.11±0.06	0.17±0.12	:	0.65±0.17	:	:	:	:	0.06±0.03
	scutellars (ant.)	:	0.03±0.03	:	:	:	:	:	:	:
	scutellars (post.)	:	:	:	:	:	:	:	:	:
	coxals	:	:	:	:	:	:	:	:	:
	ventrals	:	:	:	:	:	:	:	:	:
4	verticals (int.)	2.00±0.00	2.00±0.00	2.00±0.00	2.00±0.00	2.07±0.05	2.00±0.00	2.00±0.00	2.00±0.00	2.02±0.04
	dorsocentrals (post.)	2.00±0.00	2.00±0.04	2.07±0.07	2.00±0.00	2.00±0.00	2.13±0.08	2.00±0.00	2.17±0.08	1.92±0.07
	sternopleurals (post.)	1.89±0.06	1.97±0.03	1.86±0.07	1.94±0.06	1.96±0.04	2.00±0.00	2.00±0.00	1.80±0.09	1.88±0.06
	dors. thor. micro.	+	+	+	+	+	+	+	+	+

* : designates 0.00±0.00. 1. Differentially reduced. 2. Non-differentially reduced. 3. Maximally reduced. 4. Minimally reduced.

second being greater. The reduction of the posterior notopleurals was in the same order, although here the differences between scute-S1 and scute-L8 were not significant. The anterior sternopleurals show the above order of reduction very distinctly. The three groups of sternitals, all of which were in all three types reduced to less than half their normal frequencies, again show very distinctly the above order of reduction (scute-4 > scute-S1 > scute-L8—where by the sign > we mean "has more bristles than." The most obvious differences that could be seen on inspection were to be found in the microchaetes and macrochaetes of the dorsal surface of the abdomen. These were hardly affected by scute-4, the microchaetes appearing normal and only a few of the macrochaetes being absent. In the other two scutes the abdominal macrochaetes were almost entirely lacking and fewer than half of the abdominal microchaetes were present.

It may be added that the occasional nicking of the wing which had been observable in the original scute-S1 stock did not occur in the reconstructed scute-S1 stock isogenic with those of scute-4, scute-L8, etc. Unless this nicking was suppressed by the presence of the gene for miniature or by some unsuspected mutant gene in the isogenic stock, it had therefore been an expression of some genetic abnormality not associated with the scute-producing inversion (but perhaps associated with the smaller inversion).

Mean effects of the left ends of the inversions

The average numbers of the different bristles in the various classes of males having recombinations of the ends of the inversions are given in the last six columns of table 1. Further consideration of these may be postponed, however, until we have made a more generalized comparison of the effects of the different left ends and also of the different right ends. To facilitate such a comparison, tables 2 and 3 have been constructed. In table 2 we have averaged together the results from all three classes of flies having the left end of each given inversion. Thus, for determining the effect of the left end of the scute-4 inversion we have averaged the results from the chromosomes having the composition *sc-4L·sc-4R*, *sc-4L·sc-S1R* and *sc-4L·sc-L8R*. In this way we have eliminated, so far as possible, differential effects of the right ends of the inversions on the expression of the scute character, leaving for comparison the effects of the left ends in the material as a whole.

Examination of the more significant rows (the first ten) of table 2, representing the bristles showing differential reduction in frequency, shows that these three different left ends are responsible for considerable differences in the expression of scute, differences usually comparable in magnitude to those shown in the first three columns of table 1, where the

TABLE 2

Frequencies for different left ends (with results for different right ends averaged together).

CLASSES OF BRISTLES	BRISTLES	LEFT END OF SC-4	LEFT END OF SC-S1	LEFT END OF SC-L8
	dorsocentrals (ant.)	1.99±0.01	1.90±0.04	1.54±0.06
	supraalars (int.)	1.97±0.02	1.85±0.04	1.60±0.04
	postalars (ext.)	1.82±0.05	1.45±0.08	1.13±0.07
	notopleurals (post.)	1.96±0.02	1.08±0.10	0.54±0.06
1	sternopleurals (ant.)	1.90±0.04	1.44±0.08	0.90±0.08
	sternitals (seg. 2)	4.80±0.10	2.66±0.14	2.03±0.12
	sternitals (seg. 3)	5.01±0.10	2.66±0.14	2.08±0.12
	sternitals (seg. 4)	5.01±0.11	2.84±0.15	2.29±0.11
	dor. ab. mi.	—	=	=
	dor. ab. ma.	=	≡	≡
	ocellars	1.60±0.08	1.71±0.08	1.60±0.06
	sternopleurals (ma.)	0.16±0.05	0.21±0.06	0.12±0.04
2	sternopleurals (mi.)	10.47±0.16	10.70±0.08	10.26±0.12
	orbitals (1st)	0.06±0.03	0.03±0.02	0.03±0.01
	orbitals (2nd)	0.41±0.08	0.08±0.03	0.05±0.01
	orbitals (3rd)	0.01±0.01	0.06±0.03	:
	postverticals	0.01±0.01	0.04±0.02	:
	verticals (ext.)	:*	:	:
	supraalars (ext.)	:	0.04±0.02	:
	postalars (int.)	:	:	:
3	presuturals	0.10±0.04	0.07±0.04	:
	humerals (dors.)	:	:	:
	humerals (vent.)	:	:	:
	notopleurals (ant.)	0.19±0.05	0.08±0.04	0.03±0.02
	scutellars (ant.)	:	0.01±0.01	:
	scutellars (post.)	:	:	:
	coxals	:	:	:
	ventrals	:	:	:
	verticals (int.)	2.03±0.02	2.00±0.00	2.01±0.02
4	dorsocentrals (post.)	2.00±0.00	2.03±0.03	2.03±0.04
	sternopleurals (post.)	1.93±0.03	1.99±0.01	1.85±0.04
	dor. thor. micro.	+	+	+

* : designates 0.00±0.00.
1. Differentially reduced.
2. Non-diffly. reduced.
3. Maximally reduced.
4. Minimally reduced.

individuals have the original inversions and hence differ in regard to both ends at once.

Moreover, as in the case of the whole original inversions, but with even greater regularity, whenever significant differences in bristle number

occur the left end of scute-4 produces the least deviation from normal, the left end of scute-S1 an intermediate amount of deviation, and that of scute-L8 the greatest. Thus, so far as the left ends alone are concerned, as well as, with less regularity, for the original inversions as wholes, scute-L8 is the most extreme allele and scute-4 the least extreme. Of course, since scutes are "hypomorphic" mutations, the mutant genes have effects on the character—bristle production—similar to but weaker than those of the normal gene. This means that scute-4 really has the strongest effect—the most normal—and scute-L8 the weakest, on the developmental processes whereby the bristles are formed, although by a kind of inversion of speech scute-4 might be referred to as the "weakest allele" (in the sense of giving the least difference from the normal) and scute-L8 as the "strongest allele."

Examining the bristle effects of the left ends in more detail, in the case of the group of bristles subject to differential reduction,[2] we see not only that, as in the case of the original inversions taken as wholes, the order of increasing reduction was scute-4, scute-S1, scute-L8, but also that the differences in all of these cases were significant. This applied to all eight types of bristles in question, namely: the anterior dorsocentrals, the internal supraalars, the external postalars, the posterior notopleurals, the anterior sternopleurals, the sternitals, and the dorsal abdominal macro- and microchaetes. These differences were on the whole even greater, and showed more regularity, than those found in the case of the original inversions, but they were throughout in the same direction.

Mean effects of the right ends

The same kind of investigation was made of the effects of the right ends of these three inversions. Table 3 was constructed in the same manner as was table 2 except that in this case the three groups of individuals having the same right ends were in each case averaged together in order to obtain as nearly as possible the average effects of the right ends alone. An examination of table 3 will show in the first place that the right ends, or factors associated with them in some of the series of the present experiments, undoubtedly affect the bristles differentially, but, secondly, that these effects are seldom nearly so strong as the differential effects of the different left ends. The bristles most strongly affected by right-end differences are the posterior notopleurals and the abdominal bristles (both dorsal and sternital), but none of these effects, even, are as marked as those of the left ends on these same bristles. Thirdly, in the case of the right ends

[2] As before noted, the second orbitals, presuturals and anterior notopleurals are exceptional in being reduced to zero or nearly zero in all but one of the nine classes of flies, but in all three of these cases this class contains the left end of the scute-4 inversion.

no regularity in the effects of the three different inversions, such as was found in the case of their left ends, is apparent. Seldom if ever do we find the same sequence in the order of decrease from normal bristle number for the right ends as for the left. Neither do we find the same sequence

TABLE 3

Frequencies for different right ends (with results from different left ends averaged together).

CLASSES OF BRISTLES	BRISTLES	RIGHT END OF SC-4	RIGHT END OF SC-S1	RIGHT END OF SC-L8
1	dorsocentrals (ant.)	1.88±0.04	1.72±0.06	1.74±0.06
	supraalars (int.)	1.84±0.05	1.76±0.04	1.74±0.05
	postalars (ext.)	1.38±0.09	1.34±0.07	1.56±0.07
	notopleurals (post.)	1.15±0.10	0.88±0.08	1.35±0.09
	sternopleurals (ant.)	1.27±0.10	1.27±0.08	1.49±0.08
	sternitals (seg. 2)	3.49±0.20	2.82±0.14	2.76±0.20
	sternitals (seg. 3)	3.51±0.19	2.96±0.14	2.83±0.21
	sternitals (seg. 4)	3.92±0.16	2.83±0.14	3.06±0.19
	dor. ab. mi.	—	=	=
	dor. ab. ma.	=	≡	≡
2	ocellars	1.76±0.06	1.61±0.06	1.55±0.08
	sternopleurals (ma.)	0.11±0.04	0.15±0.04	0.22±0.06
	sternopleurals (mi.)	10.20±0.06	10.53±0.14	10.55±0.15
3	orbitals (1st)	:*	0.05±0.02	0.04±0.02
	orbitals (2nd)	0.07±0.04	0.07±0.03	0.39±0.08
	orbitals (3rd)	0.01±0.01	0.03±0.02	0.01±0.01
	postverticals	:	0.01±0.01	0.04±0.02
	verticals (ext.)	:	:	:
	supraalars (ext.)	:	0.03±0.02	:
	postalars (int.)	:	:	:
	presuturals	:	0.04±0.02	0.10±0.04
	humerals (dors.)	:	:	:
	humerals (vent.)	:	:	:
	notopleurals (ant.)	0.04±0.02	0.20±0.05	:
	scutellars (ant.)	:	0.01±0.01	:
	scutellars (post.)	:	:	:
	coxals	:	:	:
	ventrals	:	:	:
4	verticals (int.)	2.00±0.00	2.01±0.02	2.03±0.02
	dorsocentrals (post.)	2.10±0.04	1.96±0.04	2.03±0.03
	sternopleurals (post.)	1.88±0.04	1.92±0.04	1.94±0.03
	dors. thor. mi.	+	+	+

* : designates 0.00±0.00.
1. Differentially reduced.
2. Non-diffly. reduced.
3. Maximally reduced.
4. Minimally reduced.

of bristle reduction for the different right ends when different bristles are compared. In the cases of a few bristles such as the second orbitals the order of the effects of the three inversions appears to be reversed for the right ends as compared with the left, but in most cases some order of differential reduction of bristles is to be found for the right ends quite different from that for the left, and the orders for the different bristles disagree with one another.

In more detail the results presented in table 3 show the following—limiting our consideration first to the eight types of bristles which are clearly affected differentially in more than one of the nine genetic classes of flies of table 1: The internal supraalars and the external postalars were nearly equally reduced in their frequencies although not entirely absent in all the groups with the three different right ends. The other six types of bristles were affected differentially by the three right ends. The anterior sternopleurals and the posterior notopleurals showed a somewhat greater average frequency in the males carrying the right end of scute-L8 than in those with other right ends. (The same was true of the presuturals and the second orbitals, but these bristles belong to the rather anomalous category in which only one of the nine genetic classes differed significantly from a near-zero value.) In respect to these two types of bristles, then, the two ends of the scute-L8 inversion acted as though more or less complementary to one another, that is, while the left end caused greater bristle reduction than other left ends the right end caused lesser reduction than other right ends. Here it might be imagined that the bristle reducing effect had merely been apportioned differently between left and right ends in scute-L8 as compared with the other two inversions. When, however, we compare the results for scute-4 and scute-S1, in respect of these same bristles, we find that, for the anterior sternopleurals, the differences between the effects of the right ends of the scute-4 and scute-S1 inversions were not significant, unlike what was found for the left ends of these inversions. For the posterior notopleurals, the right end of scute-S1 gave a greater reduction than that of scute-4, so that there was no question of a complementary action of these two right ends in relation to the corresponding left ends.

Moreover, quite the opposite of a complementary action was to be seen in the case of the other four bristles—the anterior dorsocentrals, the microchaetes and macrochaetes of the dorsal abdominal surface and the sternitals—for all these were most numerous in males with the right end of scute-4, just as, in studying the left ends, we had found them most numerous with the left end of this same inversion. In the groups having the other two right ends, these bristles were about equally reduced—a

result that was again at variance with the differential effects shown on these types of bristles by the left ends of scute-S1 and scute-L8.

The degree of consistency of the effects of the different left ends

Let us now examine our data with a view to determining to what extent the averages given in tables 2 and 3 really represent the relative effects produced by the different left or right ends (as the case may be) regardless of what other ends they are combined with. For example, we have seen in table 2 that (when we average together the results for different right ends) the posterior notopleurals occur in significantly higher frequency in flies with the left end of scute-4 than in those with the left end of scute-S1 and in significantly higher frequency in the latter, in turn, than in those with the left end of scute-L8, the relative numbers of posterior notopleural bristles thus giving, for the left ends of the scute inversions, the order 4>S1>L8. But although this holds true in table 2, where the results from chromosomes having all three different right ends but the same left end are averaged together, we may ask, Does this same relation hold also in the presence of each specific right end in turn? This may be determined by turning to table 1 and first comparing the results for the posterior notopleurals (row 4) in the first, sixth and eighth columns of data, all columns in which the right end is derived from scute-4. Here we find again the relation 4>S1>L8. Examining next the fourth, second and ninth columns, in which the right end of scute-S1 is present, we once more find the relation 4>S1>L8. Finally, comparing the third, fifth and seventh columns, having the right end of scute-L8, we see that here too the 4>S1>L8 rule prevails. Thus the results for the posterior notopleurals shown in table 2 represent a consistent tendency, a difference in the effects of these left ends that occurs no matter what right end is present with them, so long as the latter is held constant.

To facilitate a more general examination of the same question with reference to the effects of the different left ends, on different bristles, table 4 has been constructed. This gives the order of bristle number of the three left ends, first, as found in table 2, where the right ends were averaged, and then as found in the presence of each kind of right end considered separately, for all bristles of the differential class.

It will be seen from table 4 that in the great majority of cases the results obtained in the presence of the different right ends agree fairly well with one another in showing the same order of reduction of bristles by the different left ends in the presence of different right ends, and hence that they agree also with the table 2 averages. Of course where, in the presence of a particular right end, nearly maximal or minimal numbers (2 or 0) occur for more than one left end, this does not disprove a greater tendency

to bristle production on the part of one of these left ends than of the other, and so it cannot be taken as inconsistent with a difference shown in the results for the averages. In the case of a few bristles, however—notably the posterior dorsocentrals—there is less hindrance to production of excess bristles and here "2" cannot be regarded as definitely maximal, if (as seems likely here) an intensification of the same processes which cause the production of one bristle (instead of none) in a given limited region of the integument tends to cause, further, the production of two instead of one. But in the case of most bristles the latter process, if possible, requires

TABLE 4

Order of normality with respect to bristle production shown by different left ends.

	WITH ALL RIGHT ENDS AVERAGED TOGETHER	WITH RIGHT END OF SC-4	WITH RIGHT END OF SC-S1	WITH RIGHT END OF SC-L8	RATING AS TO AGREEMENT
dorsoc. (ant.)	$4>S1\gg L8$	$4^{*}=S1>L8$	$4>S1>L8$	$4\geqq S1\gg L8$	good
su. al. (int.)	$4>S1\gg L8$	$4>S1\geqq L8$	$4>S1>L8$	$4\geqq S1\gg L8$	good
p. al. (ext.)	$4\gg S1>L8$	$4\geqq S1\gg L8$	$4>S1\geqq L8$	$4>S1>L8$	good
nt. pl. (post.)	$4\gg S1\gg L8$	$4\gg S1\gg L8$	$4\gg S1\geqq L8$	$4\gg S1\gg L8$	excellent
st. pl. (ant.)	$4\gg S1\gg L8$	$4\gg S1>L8$	$4\gg S1>L8$	$4=S1\gg L8$	good
sterni. (2)	$4\gg S1>L8$	$4>S1\geqq L8$	$4\gg S1>L8$	$4\gg S1>L8$	good
sterni. (3)	$4\gg S1>L8$	$4\gg S1=L8$	$4\gg S1>L8$	$4\gg S1>L8$	good
sterni. (4)	$4\gg S1>L8$	$4\gg S1=L8$	$4\gg S1>L8$	$4\gg S1\geqq L8$	good
d. ab. mi.	$4>S1=L8$	$4>S1=L8$	$4>S1=L8$	$4\gg S1=L8$	excellent
d. ab. ma.	$4>S1=L8$	$4>S1=L8$	$4\gg S1>L8$	$4>S1>L8$	good

$=$ denotes equality or virtual equality of bristle number.
$\geqq$ denotes an inequality which has little statistical significance.
$>$ denotes a nearly significant or significant inequality.
$\gg$ denotes a great inequality of high statistical significance.
* Signifies that the numbers for the groups on both sides of the equality sign are maximal, and that for this reason differences in the effects of the ends in question could not be observed.

considerably more "causative pressure" than the former, except where qualitative changes are involved as an aid to it. It is also to be expected, as a result of errors of sampling, that some differences seen in averages will appear as virtual equalities in the smaller records given by the separate right ends. Bearing these considerations in mind, perusal of table 4 will show that there is on the whole a rather good agreement in the order of effectiveness of the left ends obtained in the presence of different right ends.

In fact, only the ocellars (which in general show a partial reduction but one not markedly differential) show any marked inconsistency in the results, and the results from them are hardly to be considered as being so reliable as those from the other bristles anyway. For the ocellars, unlike

the other bristles, vary not merely by presence or absence but also in size, so that it is often hard to say whether the bristle is present or not, and the values given for them are not so objective. An additional sign of their irregularity is the fact that the rather arbitrary values that have been found for the ocellars do not follow, even in the averages for the left ends, the order 4 > S1 > L8 found for all bristles which are clearly affected differentially.

For the bristles here under consideration the order 4 > S1 > L8, varied occasionally by equality of adjacent classes, was found in nearly every case, in the presence of each right end considered separately, as well as for the right ends averaged together. Thus there can be no doubt, first, that in the case of each bristle there is a general rule representing the order of effectiveness of these left ends on that bristle (regardless of the right end) and, second, that this order of effectiveness is the same for bristles of nearly all kinds.

The degree of consistency of the effects of the different right ends

The orders of frequency of the differential bristles found for the different right ends, in the presence of the different left ends, are shown in table 5, which has been constructed by the same method as table 4. It will be seen that the effects of the right ends show little if any consistency. That is, for any given bristle, the order of effectiveness of the three right ends in the presence of one left end bears little or no relation to their order in the presence of another left end, and the order shown for a given bristle in the averages (as in table 3) thus represents no real rule or general tendency for the right ends, in acting on that bristle.

It is, to be sure, not to be expected that a given gene, or, still better, group of genes, in comparison with its alleles or group of alleles, will necessarily affect the production of all bristles in the same direction, and in fact two mutant scute alleles themselves often show, in the case of different bristles, an opposite order of effectiveness. But even in such cases we should expect that, in the presence of a given modifier (corresponding, for purposes of this illustration, to a given left end), a given pair of alleles would usually have differential effects that were similar, so far as they were discernible at all, to those which it had when in the presence of another modifier. For, in the gene interactions of development, additive effects of some degree (grading into complementary effects at times) represent much the more frequent types of processes, and cases of "negative interaction" (when a given gene-difference in the presence of one allele of another locus gives an effect opposite in direction to that which it has in the presence of a different allele of the latter locus) are very rare, as is

also to be expected theoretically. Thus the great amount of disagreement (including much "negative interaction") here found would indicate that the apparently diverse effects of these right ends have in reality been caused by other factors, no doubt of a genetic nature, but independent of the right ends themselves and differing in different stocks having the same right end. Differences of this kind might exist in the more distal parts of

TABLE 5

Order of normality with respect to bristle production shown by different right ends.

	WITH ALL LEFT ENDS AVERAGED TOGETHER	WITH LEFT END OF SC-4	WITH LEFT END OF SC-S1	WITH LEFT END OF SC-L8	RATING AS TO AGREEMENT
dorsoc. (ant.)	4≧L8=S1	4=S1=L8	4=S1=L8	4≧S1≧L8	good
su. al. (int.)	4≧S1=L8	4=S1=L8	4=S1=L8	4=S1≧L8	fair
p. al. (ext.)	L8≧4=S1	L8≧S1≧4	4=L8≧S1	S1=L8=4	very bad
nt. pl. (post.)	L8≧4>S1	4*=S1=*L8	L8=4>S1	L8=S1≧4	bad
st. pl. (ant.)	L8≧4=S1	4*=S1>L8	L8>S1=4	L8=S1>4	bad
sterni. (2)	4>S1=L8	4≧S1=L8	S1=4>L8	4=S1>L8	fair
sterni. (3)	4>S1=L8	4=L8=S1	S1≧4=L8	4=S1>L8	fair
sterni. (4)	4>L8=S1	4>S1=L8	4=S1=L8	4>S1=L8	fair
d. ab. mi.	4>S1=L8	4>S1=L8	4=S1>L8	4=S1>L8	fair
d. ab. ma.	4>S1=L8	4=S1>L8	4=S1>L8	4>S1>L8	fair

(Symbols as in table 4.)
* Maximal; see footnote table 4.

the major autosomes and in the fourth chromosomes of the isogenic lines first constructed, and in the chromosomes of the inbred Amherst line to which the former were crossed.

The suspicion that it is not the differences in the right ends themselves, or at any rate not genes there derived from the right end of the original normal X chromosome, that are responsible for the phenotypic differences that are manifested so irregularly in the presence of the different right ends, is heightened by the consideration that the finding of genes influencing bristle pattern at all, in precisely this location in the right end of a normal X chromosome, would be entirely unexpected—although we might attribute the effects to genes of a hypothetical "scute complex" that have been transferred to this position from the scute region by the inversions. In the absence of other, more noticeable, effects of the differences in the right ends of the inversions, it would be strange if exactly that character, scute, which we are studying because of the known influence upon it of the different left ends, should also be capable of being affected differentially by the genes derived from the given very limited region of the right end of the X chromosome. This consideration has especial pertinence in view

of the fact that the region in question is part of the so-called "inert" or "chromocentral region," and that in large measure the chromocentral regions of other chromosomes, also, act as if homologous to it, so that most phenotypic differences which it might be responsible for, even if they did happen to concern bristle pattern, would in fact have been "covered" or obscured, in the males examined, by the presence of the Y and the other chromosome parts in question.

These objections would not apply, however, to the hypothesis that the differences in the right ends of the inversions were due to a gene or genes affecting bristle pattern that had originally been in the left end, and had formed part of some hypothetical "scute complex," as above mentioned. But even this hypothesis still suffers from the important difficulty that the supposed effects of these genes are so irregular and non-additive, and often even negative in their interaction. In addition to that, there are other difficulties, which can be better appreciated after we consider separately the two forms which this hypothesis may assume.

On the first form of the hypothesis, exactly the same amount of bristle-producing gene material has been transferred from the left end to the right by all three inversions, but differs in its effect in the three cases by reason of the "position effect" of the differences in gene associations, attendant upon the presence or absence, and different degree of propinquity (as well as, perhaps, arrangement with regard to each other) of bobbed, block A, and/or other, "invisible" genes, originally of the right end. If, however, we admit that the bristle-producing region of the original left end is thus divisible into at least three parts capable of having some effect on bristles even when separated from one another[3] (namely, "achaete," "scute," and the genetic material in question that became transferred to the right end), it would seem arbitrary to assume that the divisibility stopped with this, and so it would not appear improbable that the amount of bristle-producing material, capable of autonomous action on bristles, which was transferred from left to right end, differed in the different cases, and that these differences resulted in a part at least of the apparent phenotypic differences between the right ends.

The latter would represent the second form of the hypothesis for accounting for the right-end differences as representing real effects of the present right-ends themselves. This form of the hypothesis, however, suffers from another important difficulty, besides that of the irregular, non-additive character of the effects already mentioned. That is, there

[3] This is already partially implied by the explanation of the scute mutations as position effects involving the separation of scute from genes to the right of it, but this conception alone does not require the gene or genes to the right to be able to act autonomously on bristles, that is, when removed from the propinquity of the scute locus itself.

should in this case be some tendency to a complementary action of the left and right ends of a given inversion on the production of bristles, for genes for bristle production placed at the right end of a given inversion would necessarily have been taken away from the left end of the same chromosome. As we have seen, however, no such tendency is observable. For example, although the left end of scute-4 is associated with more bristle production than any other left end, the right end of scute-4 does not tend to produce fewer bristles than other right ends, but, in the case of three of the five types of bristles that seem to be affected differentially by the right ends, its effect too is to produce more bristles than other right ends. Moreover, a scheme of the kind in question would lead us to expect that certain combinations should result in maximal bristle production and the complementary classes in minimal, and this too is not found to be the case.

All this does not mean that the hypothesis of a highly divisible "scute complex" is necessarily wrong, but that it is gratuitous in this connection since it will not help to explain the present results concerning the right ends. If it does hold, it must be concluded that probably the elements in the right-hand portion of the scute complex function little or not at all when removed from the main portion to the left of them. In that case, the apparent differences shown by the right-hand ends are due to extraneous genes, only casually associated with the right ends. Thus these differences would not serve as evidence for the divisibility hypothesis, which would have to be founded upon other grounds. And even the first form of the hypothesis, according to which there is only one main separable scute locus (at least, only one of autonomous action) to the right of "scute proper," which gives different right-end effects as a result of the different position effects of bobbed, Block A, etc., on this locus, meets with the difficulty of the irregularity of these effects, and so becomes less probable than the alternative view, that of extraneous factors. It is hoped that a test for the latter factors may be made at some later date by a repetition of our observations on somewhat different "isogenic" stocks of the nine scutes.

THE INCIPIENT GENETIC ISOLATION OF THE SCUTE-4 AND SCUTE-L8 LINES

In the examination of the recombination classes produced by crossing over between the original scutes, an interesting finding concerning fertility was made. It was found that all males tested (ca. 50) of one of these crossover classes—that having the left end of the scute-4 inversion and the right end of scute-L8—were completely sterile, although those of all three original inversions and of all the five other recombination types were fertile. This means that the original mutants, scute-4 and scute-L8, each

contain a different mutant gene, or closely linked combination of genes, which in its original setting, and probably also in an otherwise normal genotype, does not cause sterility, but which, in combination with the gene lying near the other end of the other scute inversion, does act to cause sterility.

These, then, are complementary genes for sterility. They help to illustrate the mechanism of origination of genetic isolation in the evolutionary splitting of species from one another. Had these genes been dominant and autosomal, or dominant and effective in the female, the two original lines of scute would have given sterile F_1 hybrids with one another, by reason of this complementary action. As it is, on account of the recessiveness of the mutations, only a fraction of the F_2 from crosses between the two lines are sterile, so that the inter-sterility here may be considered as only "partial" or "incipient."

The fact that one at least of the complementary genes upon which this sterility depended was not associated with the breakage and reattachment points of either of the inversions emerged after the isogenic lines were obtained (F_8), for the recombination type in question, when modified by having all but the ends of its X chromosome replaced by our standard X (with the markers w^a, *m* and *car*), proved to be as fertile as any of the other scutes. This showed that the inter-sterility was not simply a result of a deficiency or duplication caused by corresponding breaks of the two scutes being in different positions. In other words, the intersterility was genic in origin, not due to the aneuploidy resulting from recombination of different structural types.

By the same token, the result illustrates the desirability of obtaining isogenic lines for the making of exact comparisons between the positions of breakage of different rearrangements, and proves that the prolonged crossings to which our lines had been subjected were not uncalled for.

SIMILARITY OF THE THREE SCUTE MUTATIONS AS EVIDENCE FOR THE POSITION EFFECT

The phenotypic effects of some dozen different "scute" mutations, including among them scute-4, have been carefully studied and recorded by the Moscow geneticists, under the leadership of SEREBROVSKY. All of these were shown to give distinctly different patterns of bristle reduction, and although isogenic lines were not constructed, the amount of agreement found in different lines of the same allele was, in general, enough to show that the differences found between the different scute mutations were not, in the main, due to "modifying factors." Among these twelve "scute" mutations and others studied since that time, the only ones which, like scute-4, have been found to cause a marked reduction of practi-

cally all the so-called "scute" bristles and no or a much lesser reduction of the "achaete" bristles are scute-S1 and scute-L8. Moreover, the relative amounts of reduction of the different bristles are very similar for these three mutations, although, as we have seen, neither the relative nor total amounts of reduction are identical.

Now the fact that the phenotypic patterns of just these two mutations, scute-S1 and scute-L8, are so very similar to that of scute-4, despite all the other scute mutations having patterns so different from them, as well as, usually, from each other, might be attributed to mere coincidence, until the chromosome structure of the various scute mutations is analyzed. These analyses, made mainly by MULLER, show that, among the various other scute mutations, some (such as scutes 1, 3, 5, 6 and 11) show no change of gene arrangement demonstrable either by genetic tests (of segregation and crossing over) or by salivary gland observation, others (such as the inversions scute-10 and scute-J1, and the insertional translocation scute-19) involve minute rearrangements of various kinds, one of the rearrangement (breakage) points of which always lies very close to the scute region, while still others involve divers kinds of rearrangements of larger sections of chromatin, although here too one of the rearrangement points always lies close to the scute region. Among the larger rearrangements there are translocations of various kinds, as in scute-2, scute-C, scute-J4 and scute-S2, and inversions.

It is clear that only the large inversions could have a chromosome structure, in the neighborhood of the scute locus, similar to that of scute-4. These may therefore be examined in more detail. They include (besides scute-4 and the other two inversions with which we have here dealt) scute-7, scute-8, scute-9, and scute-29. Of these, as the studies of MULLER in making recombinations of the chromosomes in question with one another have shown, scutes 7, 9 and 29 involve left-hand breaks close to the scute region, but lying to the right not only of the scute locus itself, but also of another gene, which itself is just to the right of scute, and the absence of which is lethal in its effect. These three left-hand breaks are in sensibly the same position as one another, but the right-hand points of breakage of all three inversions are widely different from one another, being in scute-7 between *rb* and *sn*, in scute-29 between *m* and *f*, and in scute-9 to the left of the proximal chromocentral region but between *car* and a gene which, in haploid dosage (relative to the rest of the X), results in minute bristles (Minute-*n*?). All these gene rearrangements, then, are clearly quite unlike that of scute-4. In the case of scute-8, on the other hand, there is, as in scute-4, one breakage point in the proximal chromocentral region (although it is to the right of the loci of Block A and bobbed), but the other breakage point, that close to the scute locus, is

to the left of the latter, so that this rearrangement results in quite different gene associations and sequences in the neighborhood of scute than does scute-4.

Thus we see that, of all the scute mutations the structure of which is approximately known, only scute-S1 and scute-L8 produce gene configurations in the neighborhood of the scute locus that even remotely resemble that of scute-4. But as for scute-S1 and scute-L8 themselves, their configurations are very similar to that of scute-4, both in respect to the position of the left-hand break and in respect to that of the right-hand break.

In regard to the left-hand point of breakage, it is to be noted that, of all the above mentioned scute mutations, these alone have their left-hand break lying just to the right of the locus of scute, between it and the "lethal" locus above mentioned that lies to the right of scute. And not only do these three left-hand points of breakage all agree in lying between these two genes (or genetic regions?) but, so far as can be determined by recombination tests, these two genes are adjacent to each other, and the three breaks thus lie in identical positions. If they lay in different positions in the case of any two of the chromosomes carrying these inversions, then one of the two recombination chromosomes, produced by crossing over between these two chromosomes, would contain the left end of that inversion which had the break farther to the left and the right end of that inversion which had the break farther to the right. This, then, would be deficient for the small region lying between these two breaks; hence the fact that the breaks were in different positions would be evident from the inviability or abnormal appearance of this crossover class, unless the small region in question contained genes of so little importance that their absence produced neither inviability nor abnormal morphological effects. As a matter of fact, the crossovers are, as we have seen, all comparable in their morphology, viability, fertility, etc., with the three original scute inversions, when the lines are made isogenic.

In this connection, the question might again be raised as to whether or not the differences in bristle frequency seen in some of the recombinational as compared with the three original inversions could in part be expressions of small deficiencies of this kind, for genes (or "sub-genes") that mainly affect bristle pattern. It could further be argued that the effects of such genes on bristle production, and even on viability, might be greater than they might seem to be at first sight, inasmuch as the scute "mutations" themselves had already caused so much reduction of bristles and of viability, of an associated kind, that further effects of the same general sort would not be very evident. All this involves questions to which we must return later. But, whatever the answer to them may be, it is clear that no genes important for life or for the production of a normal morpho-

logical appearance, except, possibly, genes or "sub-genes" connected with bristle pattern itself, can lie between the left-hand points of breakage of scutes-4, S1 and L8.

In regard to the right-hand points of breakage of the three scutes in question, it is to be observed that, as salivary gland observations have shown, all are located within the so-called "inert region," better called the chromocentral or heterochromatic region, of the proximal end of the X chromosome, to the left of the centromere. It is known that genes transplanted into or near to a chromocentral region are particularly apt to undergo changes in their expression associated with their change in position. The salivary observations, combined with genetic and cytological tests (the latter on metaphase chromosomes) of the crossovers between our three inversions have shown that, although these three right-hand points of breakage all lay very near together, and within the chromocentral region, they were all in slightly different positions, that of scute-4 lying to the right of a part of the chromocentral region but to the left of both bobbed and "block A" (the gene responsible for producing most of the metaphase chromatin of this region—see MULLER, RAFFEL, GERSHENSON and PROKOFYEVA 1937), that of scute-L8 lying to the right of bobbed but to the left of block A (a configuration that had probably involved in addition a minute rearrangement of the latter two genes with respect to each other), and that of scute-S1 lying to the right of both these genes. These genes are known to be extremely close together, however, so that the gene associations finally obtaining in the scute region of the reconstituted chromosomes must have been very similar in all three cases, far more alike than any of them was like that of any other known scute, and than any other known scute rearrangements were like one another. Moreover, other results agree with these in indicating that, for a given locus subject to manifold kinds of changes in expression (such as the loci of white, brown and scute), there tends to be a considerable similarity between the changes associated with its transplantation to the neighborhood of a chromocentral region, even though the exact location of the reattachment point may differ somewhat from case to case. This observation, however, is anticipating our argument somewhat.

The important fact for us to note at this point is that, of all the numerous and diverse scute mutations known besides scute-4, it is just those two scute mutations (S1 and L8) phenotypically most closely resembling scute-4 and each other, which at the same time are by far the most like scute-4, and like each other, in regard to their content and arrangement of genes in the neighborhood of the scute locus. This coincidence is a far too special one to be attributed to chance. Yet it must be chance factors, such as just what path given electrons will take and just which chromo-

nemas later happen to approach one another, that determine what new arrangement of genes will emerge from a given irradiation. Now if the kind of phenotypic change concomitantly produced is not a secondary matter, resulting from the kind of gene rearrangement, it cannot plausibly be conceived as having its nature fixed by just those same factors of chromonema movement, etc., which determined just what anatomy the new gene rearrangement should have. Hence the correspondence between the phenotypic similarities of the three scute mutations in question and their similarities of gene rearrangement in the scute region constitute strong evidence of the dependence of their phenotypic expression upon their type of rearrangement. That is to say, the change in expression of these genes in the scute region must be an effect of the change in positions of the genes relative to one another—the phenomenon known as "position effect."

Though not hitherto published in detail, the above was the line of reasoning followed by MULLER and PROKOFYEVA (1934) in reaching the conclusion that scute-4 and scute-L8 were expressions of the "position effect." At that time, however, these mutations had not been studied in detail and scute-S1 was not yet known; the present work, then, supports their conclusion. It may be recalled that this conclusion of MULLER and PROKOFYEVA, which implied that, in the case of the two scute rearrangements in question, "gene mutations" separate from the rearrangements but of simultaneous origin with them had not occurred, had raised presumptive grounds for going further, to the conclusion that the numerous other mutations that accompanied demonstrable rearrangements of the scute region were likewise only expressions of the changes in arrangement, and that a similar process was at work in the case of other loci as well. And, after the discovery of minute rearrangements (MULLER, PROKOFYEVA and RAFFEL 1935) the suspicion even arose that possibly the same phenomenon might even be at work in many (or all?) of the cases where there was no rearrangement large enough to be cytologically or genetically detectable as such. Such an extension of the "position-effect" interpretation of the phenotypic changes, at least to the other cases of demonstrable rearrangements, fitted in with a considerable amount of evidence from other sources, obtained by various workers at about the same time.

THE POSITION EFFECT AS A POSSIBLE CAUSE OF THE OBSERVED DIFFERENCES

Accepting the interpretation that the great phenotypic similarity of the three scutes in question is an expression of the extreme similarity of their gene arrangements, it would seem but logical to attribute those

phenotypic differences which do exist between them to the fact that their gene arrangements are not quite identical. We have seen that all three right-hand breaks differed from one another in their positions with respect to "block A" and the normal allele of bobbed. This would cause the gene groups of both the left and right ends of all three inversions to differ from one another in respect to the presence or absence of these two genes, and so it would provide a basis, through their position effects, for the observed phenotypic differences which undoubtedly distinguish the left ends, and also for any which may really distinguish the right ends of the inversions, although, as we have seen, it is possible that the differences found between the latter may really not be attributable to the right ends at all.

It might be contended that the possibility has not yet been ruled out of the existence of other genes (or gene parts?), besides bobbed and block A, lying between either the different left-hand or the different right-hand points of breakage (or some in each position), and that these other genes may be responsible, in part at least, for the observed phenotypic differences. If this is true, however, these other genes must, all told, occupy an exceedingly small length of the chromosome thread. For PROKOFYEVA'S parallel cytological studies of the salivary chromosomes have shown no perceptible difference between the locations of the three left-hand breaks, all lying at sensibly the same place within the heavy scute-containing band-complex, and they have shown further that the right-hand breaks differ in position by only one or two of the faint lines of the chromocentral region.

The hypothesis of other genes than those already detected (bobbed and block A), lying between either the right or the left-hand breakage points, would also imply that the genetic method used for detecting the existence of genes between the breakage points had not been sufficiently delicate to reveal all of them. This possibility cannot be denied, for it must be recalled that the method discloses the existence only of such genes (or gene-complexes) the deficiency of which causes lethal effects or externally distinguishable morphological abnormalities.

The essence of the method is to obtain both complementary types of recombinations between each two of the gene arrangements to be compared (in these cases, both crossovers between the inversions), and to note in each case which, if either, of these two types is lethal or abnormal. If two breaks are not in just the same position, that recombinant (crossover) having the portions of chromosome to the left of the break that lies farther to the left, and to the right of the break that lies farther to the right, will necessarily be deficient for the small region between the two points of breakage, and if the deficiency for these genes causes detectable abnormality or inviability, the fact that a gene or gene-complex normally

lies between the two points of breakage (and hence too the fact that the first break really is to the left of the second) will thereby be revealed. But not all genes are indispensable for life, that is, not all deficiencies are lethal (see MULLER 1935, PANSHIN 1938), nor is there any reason to suppose that all which are not lethal necessarily cause readily visible abnormalities. And yet such "invisible genes" might, theoretically, be as active as any others in exerting a "position effect" upon neighboring genes, and so might be held responsible for the differences found between the phenotypic effects of the left ends of the scutes here studied—and also between those of the right ends, if we grant the existence of genes affecting bristle pattern at the right end of these inverted chromosomes at all.

In the case of the right-hand breaks of the three scutes here studied, there is an especially strong possibility that other genes than the two discovered may have lain between the positions of breakage. For the region here in question, the so-called "inert" or chromocentral region of the proximal end of the X chromosome, as before noted, is known to be homologous, at least in part, with the Y chromosome, and probably with chromocentral regions of other chromosomes as well. Hence deficiencies of genes in this region would more often be "covered" by the presence of normal alleles of them in the other chromocentral regions, and would accordingly be less apt to be recognizable through the causation of inviability or abnormality. Moreover, it is found empirically (whether or not the above be the reason for it) that deficiencies of chromocentral regions do in fact have less tendency than those of other regions to cause detectable morphological or lethal effects.

To avoid possible confusion here we may point out that while, previously, we used this as an argument against a direct (non-positional) effect of genes from the right end on bristles, we are here using it to show that such genes, undetected because of the lack of such direct effect on bristles or other characters, could nevertheless be present and influence the bristles through their position effects upon the genes, derived from the left end, which especially affect bristle pattern.

The consideration last mentioned does not, of course, apply to the left-hand breaks unless we choose to regard the scute region as one in which one or more extremely minute "repeats" (duplications), closely adjoining one another and similar to but much smaller than that found in the mutant bar eye, have occurred in the relatively recent evolutionary history; for such duplications would tend to have a "covering" effect on deficiencies, similar to that existing in the case of chromocentral regions. The similarity in function of the neighboring loci of achaete and scute makes it likely (MULLER 1935b, SEREBROVSKY 1938), that there has at some time been some duplication of this kind, but as achaete is to the left

of scute and these breaks to the right of it, additional duplications would have to be postulated for our cases, if we would suppose the existence between the different left-hand breaks of undetected genes, covered, or partially covered, in the above manner. It is quite conceivable, however, that even if there were no duplications having such a "covering" action, the deficiencies might have had effects too small to have been revealed by the methods used—especially if we indulged in speculation to the effect that deficiencies might be of lesser length than that commonly envisaged as the probable (but, more correctly, the estimated maximal) length of a gene.

On either the supposition of minute duplications involving scute, or the related one of different positions of breakage within lesser lengths than that attributed to the scute gene or gene-complex as a whole, differences in bristle production could be brought about not only by the position effect of such minute parts, as ordinarily conceived, but also in a more direct way, by the presence or absence of these parts. For this and other reasons, a proper evaluation of the meaning of our results now involves us in considerations of the degree of divisibility of the genetic material, the criteria by which we recognize the presence of genes and their spatial or numerical limits, and the extent to which the string of genes may be regarded as discontinuous or continuous in structure and functioning.

CONSIDERATIONS CONCERNING THE DIVISIBILITY OF THE GENETIC MATERIAL

It may be recalled that all the methods of estimating gene number and size have admittedly given merely a minimal figure for the former and a maximal for the latter, with no indication of what the limit may be in the other direction (MULLER 1926). The latest method (MULLER and PROKOFYEVA 1934), based on estimating the minimum number of different positions of breakage in a minute, circumscribed region of a chromosome, uses the genetic method referred to in the fourth preceding paragraph to define whether or not the breaks considered are in "sensibly the same" position. Since it suffers from the difficulty above explained, of failing to detect any genes (or gene-complexes or gene-parts) a deficiency of which would not result in lethality or readily detectable abnormality, it gives only a minimal number for the positions of breakage, and consequently for the number of genes, and a maximal gene size (though the former is considerably larger and the latter considerably smaller than any figure hitherto arrived at by other legitimate methods).

Although there is nowadays little practical disagreement among geneticists as to what constitute the criteria for distinguishing the gene material, or genetic material, from other material of the cell (the definition

involving the concept of self-determination of its own characteristics by the mother material in the reproduction of the daughter material, together with a mutability that does not interfere with this property), no such understanding has been arrived at concerning the question of how the limits of *a* gene, as distinguished from its neighbor genes, shall be defined. In genetic theory, genes have been considered as (1) crossover units—hypothetical segments within which crossing over does not occur; (2) breakage units—again hypothetical segments within which chromosome breakage and reattachment do not occur (at any rate, not without destruction of one or both fragments); (3) mutational and functional units—those minute regions of the chromosomes, changes within one part of which may be so connected with changes in the functioning of the rest of that region as to give rise to the phenomenon of (multiple) allelism; or (4) reproductive units—the smallest blocks into which, theoretically, the gene-string could be divided without loss of the power of self-reproduction of any part. A category of auto-attractive units might also be added.

Although it seems often to have been assumed, there is as yet no empiric evidence, and only doubtful theoretical ground, for assuming that the lines of demarcation between genes, as defined on any one of these systems, would coincide with those on any of the others, or even for assuming, in the case of some given one of these systems (especially the mutational one) that such lines of demarcation are necessarily invariable, non-overlapping, well defined and absolute. The minimal gene numbers and maximal gene sizes hitherto arrived at have to be considered in relation to the particular method used in arriving at them, one value having been a limit obtained through crossover studies, another through studies of mutation (allelism), and the most recent one through breakage studies. Hence they do not necessarily represent the same thing, even though it might be simplest to assume that they did.

In view of the above considerations, it is not far fetched to imagine that the "gene for scute," as recognized by the test of allelism of its mutations, may nevertheless consist of an undetermined number of parts, some of which may become separated from others by breakage without such separation resulting in the loss of the reproductive power of any of these parts (although, perhaps, interfering with their functioning). These parts, then, might themselves be denoted "genes," and the whole a "gene-complex," or the parts might be called "sub-genes" or something equivalent, and the whole a "gene," depending upon the taste of the writer and upon the criterion which he prefers (that of "allelism" or that of breakage) for defining the limits of a "gene."

In fact, in the case of achaete and scute, we do have a situation verging on that above imagined, inasmuch as, firstly (contrary to the earlier belief

of the workers on scute mutations), a recessive mutant gene, or a loss, at either one of these loci, does, although only in slight degree, act as an allele of such a condition at the other locus, both in respect to the achaete and to the scute characters, and, secondly, some "gene mutations," such as scute-3, affect both characters strongly, and act as simultaneous alleles of mutants of both types; yet both breakage tests (SEREBROVSKY and KAMSHILOV (unpubl.), and possible evidence by AGOL 1931) and crossover tests (DUBININ, SOKOLOV, and TINIAKOV, 1937) have shown the "genes" of achaete and scute to be separate in both the latter senses. Since we must thus admit that the original "scute gene," as defined by the mutational test, is really divisible into two parts, "achaete" and "scute" proper, as defined both by the tests of breakage and of crossing over, we should exercise caution before denying that the remaining "scute" proper may be further divisible, even though the parts of it might not have nearly as completely differentiated functions as the earlier postulated "sub-genes" were thought to have.

It was in fact in the hope of throwing light on the above question, more than on any other, that our series of studies on scute, of which the present one forms a part, were undertaken. Among the results of the series of studies was the finding that, of thirteen breaks at or near scute that were examined with reference to each other by the method previously referred to, of recombining the portions to the left and right of the breakage points, there were in all only four distinguishable positions of breakage—distinguishable by the criterion of having between them genes (or genetic material) the deficiency of which caused lethality or some readily distinguishable abnormality. Five of these breaks are to the left of scute: two of these—*y3P* and the left break of scute-19—to the left of both achaete and yellow; two others—*y4* and *y5*—to the left of achaete and possibly of yellow; and one—*sc-8*—between the genes achaete and scute. The three breaks here studied in detail (those of scutes 4, S1 and L8) appear to be immediately to the right of scute. And the remaining five breaks—those of scutes 7, 9, 29, and S2, and the right break of scute-19—are still farther to the right, as recombinants having the part of the X chromosome to the right of one of the latter breaks and that to the left of one of the three here studied were found to be lethal.

It hardly seems possible that it should be a coincidence that all three rearrangements having one breakage immediately to the right of scute, as in scute-4, should have had their other breakage in the "inert region" of the X, to which the scute region accordingly became attached, while all five having the breakage of the scute region located farther to the right (to the right also of the right-hand "lethal locus") should have had their scute region attached to some other, non-chromocentral, region. It seems

difficult to avoid the conclusion that other kinds of reattachments would have resulted in position effects that were either invisible or dominant lethal, and so escaped detection.

As cytological examinations of PROKOFYEVA have proved most clearly in the case of scute-19, all these breaks, and hence the genes between them, lie within a limited region of the second conspicuous band, or rather, band-complex, found in passing rightwards along the salivary X chromosome (in the region designated as 1 B 1 and 2 in BRIDGES (1938) diagrams). The most detailed studies have shown that not more than two entire bands, as seen with visible light, can lie between the leftmost and the rightmost of our breakage points (that is, between the left and right breakage points of scute-19), and the ultraviolet photographs of ELLENGORN (ELLENGORN, PROKOFYEVA and MULLER 1935) have shown not more than three bands. If we regard yellow and achaete as separate loci, and then take into consideration the evidence showing four different breakage points, we must conclude that there are at least four genes in this tiny region, hence more genes than detectable bands.

Is it however, legitimate to infer that there are only four genes, in the sense of independently reproducible segments of genetic material, separable by breakage, within this space? The finding that all the 13 breaks seemed to fall into just four positions instead of 13 would, to be sure, seem to show a considerable limitation in the number of possible breakage points here. But, when the method of determining whether or not two breakage points are in different positions is taken into consideration, it must be admitted that smaller differences in position might conceivably have escaped detection. For, in view of the fact that the deficiency of both yellow and achaete together was found not to be lethal, and that the deficiency of "scute" itself (that is, of the region between our second and third distinguishable positions of breakage) is not lethal in quite 100 percent of cases, it would be strange if yet smaller deficiencies were not more viable.

If these smaller deficiencies involve genes or "subgenes" that form part of the hypothesized scute complex and have their morphological expression mainly in bristle pattern, it might be thought that it should have been possible to detect them by reason of the relative absence of bristles in one of the classes of recombinants (the deficient class) as compared with the other classes. Yet, as we have seen, there was no evidence of such complementary action. There are, however, several circumstances that might well interfere with the detection of such subsidiary parts by this means. In the first place it might be that portions of the complex, when removed from some chief grouping, came, through a species of position effect, to have little or no influence upon the character in question (here, bristle frequen-

cy); thus the really deficient class might show little or no more evidence of the deficiency than one of the original classes, in which the parts were all present, but merely separated. Secondly, we know, as a matter of fact, that these rearrangements have somehow brought about a drastic reduction of effectiveness (in bristle production) of the scute gene or scute complex taken as a whole, and in this state of very low bristle frequency (involving the total absence of many bristles ordinarily very responsive to variable influences), slighter influences, due to the presence or absence of some weakly acting minor part of the whole, might well escape detection. Under these circumstances, even quantitative phenotypic studies on truly isogenic material might fail to give evidence of the gene complex having been divided. The locus of yellow affords an even better illustration of this principle than does that of scute, for in the presence of the inversions called yellow 4 and 5 the character-effect of the locus is completely absent (the flies being completely yellow, as when the gene itself is absent); and so, far from telling (by our method of the recombination of rearrangements) whether the breaks in question may lie within some yellow gene-complex, it is impossible even to know whether they are just to the right or just to the left of it, or whether, perhaps, a break had occurred on both sides simultaneously, with resultant actual loss of the gene.

In the case of the scute rearrangements, we have seen that, although the apparent differences in effects of the right ends of the inversions do at first sight suggest that they contain parts of a scute complex, which had been transplanted to these positions by the process of breakage and reattachment, nevertheless the irregularity of the same effects, and the lack of any tendency for the effects of the right and left ends to be complementary, destroy the usefulness of such an interpretation of them and make it more likely that these differences have had no connection with the nature of the rearrangements. On the other hand, the rather regular differences shown by the left ends can, *a priori*, be interpreted equally well as due to differences of position effect caused by the presence or absence of bobbed, block *A*, and/or another, invisible gene, or genes, from either the right or left ends or both, that have in the main other functions than those involved in the "scute" character itself; or as due to the separation from the scute gene or gene-complex of relatively minor parts of it which function little or not at all when separated from the rest of it, but which when in the neighborhood of the latter, through their interaction with it result in a considerable increase of bristle production, involving different bristles to differing degrees. It should be observed that these two hypotheses really run into one another, for by admitting, on the former hypothesis, more and more independently reproducible genes or gene-parts, derived from the left end, and by more and more reducing their action, when in their orig-

inal position, to that concerned with the pattern of the bristles, we gradually arrive at the second hypothesis.

The real question here, then, is to what extent there may exist, in the scute region of the normal X chromosome, a series of elements that can be separated from one another, without losing their capability of reproduction, and that, at least when in their normal positions, co-operate in the production of the given character (the bristles produced by the normal allele of the scutes). Still another question is that of the possible existence of elements, not capable of reproduction except in their original setting, that are subject to being cut off from their reproductive base by chromosome breakage. It may also be questioned to what extent the functions of these elements differ from one another (as, for instance, to what extent they may affect different bristles), and to what extent each of them may subserve other, differing characters as well. Certainly the "gene" for achaete, just to the left of scute, has some part in this scute complex. And our series of studies on the chromosomal conformation of the other alleles shows that, just to the right of the main scute locus also, there lie one or more genes which (as compared with genes that may be substituted for them by inversion or translocation of other euchromatic regions) exert an appreciable position effect assisting in the production of the normal ("non-scute") bristle characters. At least one of these genes is not immediately adjoining the scute locus but lies to the right of the locus of the lethal which itself is to the right of scute, because the scute character is affected even when the chromosome is broken and rearranged to the right of this lethal locus. We cannot, at present, be sure that between the boundaries so set, or even within the main "scute locus" itself, the genetic material is not still further divisible, in the sense defined, nor know to what an extent it may be thus divisible. Certainly neither our experiments here presented, nor our whole series of studies on scute, give good evidence for the existence of such further divisibility, but it is equally important to note that they do not give convincing evidence against it either, as they might seem to do on first consideration, and, in fact, it is quite possible that a part of the effects here noted are results of such divisibility.

We have laid emphasis on this matter because this question of the further divisibility (without loss of reproductive capacity of the elements) of regions of the chromosome that have, on other grounds, been considered as probably representing single "genes," has recently been brought to the fore by various results in the field of gene mutation and chromosome rearrangement. In the first report of the finding of minute rearrangements, and of the phenotypic changes accompanying them (MULLER, PROKOFYEVA and RAFFEL, 1935), the suggestion was made that many, perhaps all, of the supposed gene mutations produced by irradiation, and probably some

at least of the spontaneous ones as well, represented fundamentally the same phenomenon, although on an even smaller scale than that found in our cytologically demonstrable cases. But at that time we did not believe that gene mutations in general—especially those forming the basis of evolutionary progression—could be of such type, because we did not think of "genes" (as defined by the mutational properties of a given chromosome region) as being further genetically divisible, and we realized that the number of kinds of somatic changes dependent upon the limited position effects of rearranging these relatively large and few units could not be nearly of the order of magnitude necessary to provide for the great evolutionary plasticity and diversity of organisms. Since then, however, considerations of the similarity in expression, and in mode of production, of "gene mutations" and of minute rearrangements, and of the empirical impossibility of drawing a line between them, have led us to question whether or not there may be even minuter rearrangements than those which change the position of "whole genes" (as taken in the older, less flexible sense). For if smaller, linearly arranged parts of those regions which have been considered as genes (by criteria of mutation and allelism) may become rearranged with respect to each other in essentially the same manner as the "whole genes," then, at least by having such parts rather numerous and of a number of diverse kinds, sufficient plasticity of result is introduced to make plausible the conception that most gene mutations might be of this kind.

Among the findings which played an important part in leading us to put the present question, was that concerning the effect of varying the dosage of radiation upon the production of mutations. It had been found by MULLER and various co-workers (KOERNER, VOGT, BELGOVSKY, BERG, PANSHIN and BORISOFF), and later confirmed by certain results of DUBININ and his colleagues, that the frequency of rearrangements that are readily detectable as such genetically, that is, "large rearrangements" induced by X rays, is proportional to more than the first power of the dosage (ionizing capacity) of the rays—the value being, for the doses ordinarily used, about half-way between the first and second power. On the other hand, as various workers have shown (OLIVER, HANSON, TIMOFÉEFF-RESSOVSKY and others), and as special studies of RAFFEL have recently confirmed, the frequency of the "gene mutations" is simply proportional to the first power of the dosage of radiation. Here then there seemed to be a rather fundamental distinction between the mechanism of production of rearrangements, which presumably always involve at least two breaks and so may depend more nearly on the second power of the number of ions, and "gene mutations," which conceivably involved (at least in their initial stages) but one chemical substitution.

The possibility had not yet been excluded, however, that *minute* rearrangements might obey the same dosage rule as gene mutations, for they had not yet been tested in relation to this question, on account of the difficulty usually encountered in obtaining enough recognizable cases of them. Studies of BELGOVSKY, PROKOFYEVA, RAFFEL and MULLER, however, showed that, by the use of special stocks, minute rearrangements in or near certain chromocentral regions could be obtained and recognized in sufficient quantities; and it was accordingly decided that the frequency of the production of these rearrangements by different doses of X-rays should be measured. This has been done both by BELGOVSKY and by MULLER and MAKKI (see BELGOVSKY 1939, MULLER, MAKKI and SIDKY 1939, MULLER 1939) and their results have shown clearly that these minute rearrangements vary as the first power of the dosage, just like the supposed gene mutations. This result now breaks down the last empirical distinction that it was possible to draw between "gene mutations" and "chromosome rearrangements" and opens wide the door for considering the former also to result from double or multiple breaks, with reattachment, like the latter, but on an even minuter scale. And this in turn raises questions concerning the limits of what have been called "genes," and the propriety of assuming that "genes" as conceived according to our different hypothetical criteria are necessarily coextensive.

Since the above possibilities were suggested by MULLER (1937) some similar suggestions have independently been made by GOLDSCHMIDT (1937 a, b, 1938). Although we differ from GOLDSCHMIDT in that we regard our suggestions as pointing, as yet, only to possibilities, while he regards his corresponding proposals as representing something highly probable, and although we differ also in various matters of emphasis, implication, and subsidiary interpretations, as well as in what we regard as the more valid grounds for regarding such a conception as plausible, we must nevertheless agree in the more general proposition, central to his theme and ours, that the "gene," in the rather loose sense in which it has so long been taken for granted by most geneticists, may *perhaps* be genetically further divisible, even into many genetically linearly arranged portions of semi-autonomous character (autonomous in the sense of their being able to reproduce when in other linear arrangements), and that, contrariwise, neighboring "genes" often or usually co-act, in a manner made possible by their juxtaposition, so as to produce character-effects that depend upon overlapping regions, somewhat larger than a single "whole gene," as formerly conceived. The idea of a "whole gene" or, more directly, of "a gene" itself, thus comes under scrutiny, and we are at present far from having evidence that the "whole gene" or "the gene" (meaning just "*one* gene") would be the same when defined by the various different criteria listed. We should not wish,

however, to deny the alternative possibility, that the in some ways simpler-seeming conception of indivisible genes in the older sense (modified, however, by the conception of the position effect) may eventually be found to correspond to the facts, after all. What we do wish to emphasize chiefly is that more work will be needed before the question can be decided.

Whether genetics and cytology themselves will provide methods adequate for solving such remote questions it is as yet too early to say. As an example, however, of a type of genetic study that might conceivably throw light on a question of this kind, we shall mention a study undertaken by MULLER, with the aid of SINITSKAYA, to determine whether it was possible to induce reverse mutations in scutes of the type reported upon in the present paper. If these scute mutations represented position effects of the rearrangements of "whole genes," in the older sense, then it should much more readily be possible to obtain reverse rearrangements that would restore the original order, and the normal phenotype, than if the scute mutations represented rearrangements the breakages of which had come between smaller parts of the "gene." For in the latter case new breaks would much more rarely (especially when both of them were considered) come between just the same two parts as before—a condition that might well be necessary in the case of so manifestly complex a gene as that for scute. In the latter case, too, there would have been a chance that a part of the gene had been lost when the original mutation occurred, and so could not be restored. On the other hand, in case "whole genes" were concerned, there would be especial reason for hoping that exactly reverse rearrangement might be found within a reasonable time, because it is known that breaks occur much oftener in or near the chromocentral region, and the scute rearrangements here in question are, as we have seen, such that both points of reattachment now adjoin such a region.

In order to increase the chance of breakages occurring near both of the original positions at once, a recombination chromosome was used which had the right end of scute-4, which contains the longest chromocentral region to the right of its right break, and the left end of scute-L8, which contains more chromocentral region just to the right of its left-hand break than scute-4 does, since bobbed is included in this region in scute-L8 but not in scute-4. (Unfortunately at that time it was not yet known that block-A was not in this region of scute L8, along with bobbed, otherwise the left end of scute-S1, which has both these genes at the left end, would have been used.) Scute males having the above recombination chromosome were then X-rayed with a dose of about 3,000 *r*, and crossed to females with attached X chromosomes, and their sons were examined for possible reverse mutations of scute to a normal or near-normal phenotype. How-

ever, among the approximately 50,000 male offspring examined, none showed such a change.

We can by no means regard this negative result as being in itself of much significance, until the frequency with which breaks in and near such chromocentral regions occur becomes better known, and it will be desirable to have the experiment repeated, with even larger numbers. We present it only to show that the possibilities of investigating the general problems here at issue by means of genetic tests are as yet by no means exhausted.

All in all, we do not wish to give the impression that our results with the three scutes here reported upon actually support the idea of a finely divisible gene. They do, however, give evidence for the position effect, and this phenomenon in itself connotes that the gene, as defined by the mutation-allelism test, extends over a larger region than that defined by breakage, crossing over or self-reproducibility, and that, in fact, the regions of successive genes, as defined by the mutation-allelism test, do not merely adjoin but overlap each other. On the other hand, our present results do not, in themselves, give evidence of a finer divisibility than that found in our more general study of breakage in the scute region, which had indicated the existence of at least four separable genetic constituents ("genes") within the space of one or two visible bands, and which further showed that, if other breakage points than those forming these four parts existed, the elements included between them must be undetectable by ordinary genetic tests. It was, then, not these results, but those of other investigations, especially the recent ones on minute rearrangements and their relation to dosage, those on the phenotypic relations of achaete and scute, and general considerations based on the manner of production of mutations and the nature of the gene, that made it necessary to take up in some detail here the question of finer gene divisibility, and to consider to what extent our present results might be in favor of or opposed to such a conception. As we have seen, they can be interpreted in either way with roughly equal plausibility (aside from extraneous considerations that might affect the plausibility of the divisibility idea). Hence, so far as our present results are concerned, this matter must still be left an open question.

It should eventually be possible to obtain further light on this question through the obtaining of additional scute alleles and their intensive study. The next time an inversion occurs with the left break in the same apparent position as that of scutes 4, S1 and L8, and the right break just to the right or left of *bb* and block A or between them, will it, when made isogenic with whichever of these three scutes whose arrangement it seems to duplicate, also be identical with that scute phenotypically? If so, it is unlikely that the "scute gene" is subdivisible into different parts affecting the bristles. Such work is more or less complementary to the study of the effects of re-

verse rearrangement above discussed. And, in general, a continuance of intensive work on breakage in the scute region should give more information about the number of possible points of breakage.

There are other considerations bearing on the question of the segmentalism of the genetic material, for which the reader may be referred to another paper (MULLER 1940). Among these are the results, obtained by MULLER and MACKENZIE (1939) since the present paper was written, showing that ultra-violet light produces gene mutations but not gross rearrangements. This and other facts would argue for a more fundamental distinction between gene mutations and rearrangements than that above suggested as one of the alternative extremes.

SUMMARY

1. Analysis of the three scute mutations, scute-4, scute-S1 and scute-L8, produced by X-rays, shows that all three involve an inversion, having its left break close to the right of the "scute gene," and its right break in the proximal chromocentral ("inert") region of the X chromosome. None of the numerous other scute mutations investigated has breaks in both of these positions.

2. Single crossovers of both contrary classes between each of these inversions and each of the others are viable, and phenotypically similar to the original types (except as noted in section 5, following). Hence all three of the left hand breaks must have been in identical positions or else in positions so close to one another that deficiency of the region between them was neither lethal nor productive of any detected abnormality (except as noted in section 5). Salivary study by PROKOFYEVA revealed no difference in positions of the left-hand breaks.

3. Genetic testing of the crossover chromosomes for bobbed and cytological testing (in metaphase stage) for block A showed that, while in scute 4 the right-hand break was to the left of both these genes, in scute-S1 it was to the right of both of them, and in scute-L8 between them, carrying bobbed to the left end and leaving block A at the right, a result that had probably been made possible by the occurrence of a further, very minute, rearrangement, whereby these two genes interchanged their positions. If other genes differentiated the positions of the right-hand breakage points, the absence of these from the X chromosome produces no effect detectable by the methods used. Salivary study by PROKOFYEVA showed that the right-hand breaks were differentiated by only one or two faint lines of the chromocentral region.

4. Phenotypically, these three mutations, and also the recombinations between them, resemble each other, but the exact extent of the resemblance or difference could not be gauged until isogenic groups of all nine

of them (the three original combinations and the six possible recombinations between them) were constructed. The methods are given whereby, through a scheme of crosses involving 26 successive generations, the nine lines isogenic for all of the X chromosomes except their very ends were constructed, and whereby, through ten further generations, these nine groups were made isogenic for their major autosomes (again except for their very end regions) at the same time.

5. One of the recombinations between the original scutes (that having the left end of scute 4 and the right end of scute-L8) proved to be sterile, at least in the male, but when the isogenic lines were obtained the same type of recombinant, having the bulk of its X chromosome replaced by that of the standard X used, was as fertile as the other scutes. This shows that complementary genes for sterility had arisen in the X chromosomes of the two scute mutants, lying in different loci, and (at least one of them) separable from and not dependent upon the points of rearrangement of the inversions. Alone, neither caused sterility, but only in combination with one another. This serves to illustrate the mode of origination of genetic isolation in the evolutionary splitting of species.

6. Comparison of the nine isogenic groups shows them all to be much more similar to one another than any of them was to any of the numerous other investigated scute mutations. This, taken in connection with the unusual similarity of all of them to each other in gene arrangement, proves that there is a close connection between type of gene arrangement, that is, between the kind of neighbor genes a given gene possesses, and type of phenotypic effect, that is, the phenotypic similarity must be due to the "position effect" of the genes, and their change of expression, seen as the visible scute mutations, must have been due to the change of gene positions relative to their neighbor genes.

7. Despite the great similarity, there were unmistakable differences between the different types which it is logical to refer to the relatively slight differences in their gene arrangement.

8. Analytical comparisons of the nine classes and of averages between certain groups of them, showed that the left ends of the inversions differed in their effects in a consistent fashion, the order of abundance of bristles of practically all differentially affected types, in the presence of the left ends of the different scutes (with the right end derived from the same scute in all three cases), being scute-4 > S1 > L8. The isogenic lines of the original combinations of left and right ends showed the same order of effects. It is inferred that these differences in the left ends, and in the original scutes as a whole, are caused by the position effects, in connection with the scute gene, of genes that differentiate the present left ends of the chromosomes from one another, and that lie just to the right of the leftmost of these

scute breaks. Among these genes may be bobbed and block A, and possibly other "invisible genes" from the original right or left ends, or from both.

9. Although there were also some differences of statistical significance produced by the different right ends (in the presence of a constant left end), these were considerably less than the differences produced by the left ends, were not in the same direction in the case of different bristles, showed no consistency when the results from the different right ends in the presence of one left end were compared with the corresponding results in the presence of another left end, and showed no tendency to be complementary in value to the results from the left ends (as they should if due to a transfer, by the inversion, of different bristle-producing genes, or subgenes, from the original left to the right end). The interpretation accordingly suggests itself that the apparent differences obtained from the right ends might perhaps be really due to a lack of complete isogenicity on the part of the lines studied.

10. The question is raised of to what extent the breakages in the region of scute in this and other cases where they seem identical in position are really identical, or may be separated by parts of a "gene-complex" (or "gene," according to definition) which, although able to reproduce separately, function to produce bristles only, or mainly, when in proximity to one another, and function normally only when in proper arrangement. On account of the extreme reduction of bristles in these cases of nearly identical rearrangement, caused by the position effect, the influence upon the bristles of differences in such hypothetical parts might not be noticeable under these conditions. However, it is conceivable that they, rather than bobbed, block A, or other genes from the right end, are the chief differentiating factors that, by their differing position-effects with the rest of the scute complex, cause the differences that have been noted between the effects of the different left ends. Similar considerations apply to other loci, and make it uncertain to what extent our criteria as to what constitutes a single gene, based upon different methods of approach (herein listed), refer to co-extensive structures. These considerations likewise affect our judgments regarding gene size and number. The undoubted overlapping of function of portions of the chromonema distant enough to be separable by crossing over or breakage, caused by the position effect, raises further difficulties in the delimitation or definition of *a* gene. In recent publications GOLDSCHMIDT has independently expressed a point of view which is in some essential respects similar to this.

11. The finding of minute rearrangements, with position effects resembling gene mutations, has made the above question more acute, for on the hypothesis of independently reproducing gene-parts or "sub-genes" (or

genes within a larger gene-complex), the "gene mutations" *might* simply be rearrangements so small as to lie within a "gene" (or gene-complex). This would agree with the recent discovery by BELGOVSKY and MULLER that demonstrable rearrangements of minute size vary in frequency as the first power of the X-ray dosage, like "gene mutations," and differently from large rearrangements, the variation of which is, for ordinary doses, approximately as the 3/2 power of the latter. However, the question of gene delimitation is as yet far from settled, being as yet in the earliest stage—that of being formulated. Certain genetic avenues of approach to it can be descried, of which one illustration is cited, which involved an experiment, having negative results, designed to find reverse mutations of one of the recombinational scutes here studied. The failure here would argue for a relatively high divisibility of the genetic material, but is not yet to be taken as more than merely suggestive, especially since other considerations and results point in the opposite direction.

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THE ACTION OF CERTAIN MUTANTS UPON THE PENETRANCE OF HETEROZYGOUS VESTIGIAL WING IN DROSOPHILA MELANOGASTER

M. M. GREEN AND C. P. OLIVER
University of Minnesota, Minneapolis, Minnesota

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INTRODUCTION

ONE of the methods used in the study of the mechanism of gene action in *Drosophila melanogaster* has been to observe the modification of the phenotypic effects of mutant genes following alterations in the environment in which development occurs. Thus the effects upon the mutant phenotype of temperature changes, crowding of the cultures, and starvation of the developing individuals have been observed.

More recently, certain genetic and environmental factors which alter the duration and rate of development have been studied for their effects upon the mutant phenotypes. It has been found that the facet number in homozygous vestigial, Bar individuals is significantly smaller than in non-vestigial, Bar individuals (MARGOLIS 1935). Conversely, increase in the facet number of Bar individuals resulted when mutant larvae were starved (BODENSTEIN 1939). A decrease in the size of Lobe eyes occurred when certain of the dominant Minutes were introduced (DUNN and COYNE 1935). A systematic study of a number of eye and wing mutants showed an increase in the penetrance of all mutants studied when either of two dominant Minutes was introduced (BRYSON 1940). An increase in the penetrance of vestigial wings in the presence of dominigenes has been reported in flies heterozygous for vestigial and having also certain chromosomal aberrations (GOLDSCHMIDT, GARDINER, and KODANI 1939). By starving homozygous vestigial individuals, CHILD (1939) was able to observe an increase in the size of the imaginal wing. A similar effect for vestigial^{no} and cut^{n} has also been observed by BRAUN (1939).

The data presented here are the results of a study of the influence of two Minute mutations and of a duplication of the third chromosome upon the penetrance of the heterozygous vestigial wing in *Drosophila melanogaster.*

MATERIALS AND METHODS

The two dominant Minutes used here were respectively $M(3)w$ located at 80 on chromosome III, and $M(2)l^2$ located at 101 on chromosome II. Both cause a prolongation of the larval period. In the case of $M(2)l^2$ the mean prolongation of the larval period at a temperature of 25°C amounts to 12 hours (DUNN and MOSSIGE 1937). Among $M(3)w$ males the prolongation of the larval life is 41.3 hours, while among the females of this geno-

type the prolongation amounts to 42.9 hours at a temperature of 25°C. (BREHME 1939).

Duplication *T1305* females occur with a relatively high frequency (about 40 percent) when males heterozygous for *T1305*, a translocation between chromosomes I and III and marked with the dominant Dichaete, are mated to females free of the translocation. The resulting non-Dichaete females possess a duplication of an inner fragment of the right arm of chromosome III. Phenotypically, the duplication in the female produces a slight spreading of the wings, increased pigmentation of the abdomen, and, occasionally, rough eyes. Although two percent of the duplication males have nicked wings, no nicks were observed among three hundred females examined by OLIVER (1937). Unpublished data indicate that occasionally duplication females have nicked wings.

In all matings the female parents were homozygous for vestigial and were derived from a vestigial stock which had been inbred in mass culture for a period of two years. Females were mated to males $M(2)l^2$ balanced with *Cy*, or to males $M(3)w$ balanced with $In(3R)C$, or to males *T1305D*. As a control test, vestigial females were mated to wild-type males. In order to minimize any effect due to physiological differences in age, all females were selected as virgins and held for 48–72 hours. They were then mated and held in vials for 48 hours, and then transferred without etherization to half-pint milk bottles. Each culture contained approximately 50 cc of the regular banana-agar medium, enriched with brewers' yeast, and seeded with two drops of a thick yeast suspension. In order to minimize the factor of crowding, five or six pairs of parents were used to a mating, and females were allowed to oviposit only for a period of twelve hours before they were transferred to fresh bottles. The total time of oviposition of any female never exceeded sixty hours. All experiments were conducted at a temperature of 23 ± 1°C.

EXPERIMENTAL

Prolongation of the developmental period

No specific study was made upon the prolongation of the various stages of the life cycle; but in the course of the experiment, observations were made upon the length of the total life cycle of the $vg/M(2)l^2$ and $vg/+$, $M(3)w/+$ individuals. The delay as based upon the time of emergence of these individuals at 23° is in agreement with that expected on the basis of the cited experiments. It was found that the $vg/M(2)l^2$ individuals emerged approximately 18 hours later than the $vg/+$ individuals, and that the $vg/+$, $M(3)w/+$ individuals emerged approximately 60 hours later than the non-Minute sibs. These measurements are based on twelve hour egg-laying periods, and observations of emergence every twelve hours. In all

cases the males emerged later than the females. No precise measurements of the prolongation of the developmental period in duplication *T1305* were made; however, it was noted in series tested concurrently that the time of emergence was later than *vg*/+ males but earlier than the emergence of the *vg*/$M(2)l^2$ females. On the basis of these observations the compound genotypes considered here are seriated in order of increased length of developmental periods as follows: *vg*/+ ♀♀; *vg*/+ ♂♂; *vg*/+, duplication *T1305* ♀♀; *vg*/$M(2)l^2$ ♀♀; *vg*/$M(2)l^2$ ♂♂; *vg*/+, *M*(3)*w*/+ ♀♀; and *vg*/+, *M*(3)*w*/+ ♂♂.

Effects on the heterozygous vestigial wing

From the data obtained it is clearly evident that the introduction of either $M(2)l^2$, *M*(3)*w*, or the duplication *T1305* into heterozygous vestigial individuals resulted in a modification of the wing phenotype, and that the modification is expressed in the form of notching and nicking of the wings. In each case, the frequency of wing scalloping is significantly greater than in the controls.

TABLE 1

Effect of three genetic factors on the heterozygous vg phenotype. Frequencies in percent with standard error for each of three phenotypic classes. Genotypes listed in order of increased length of developmental period.

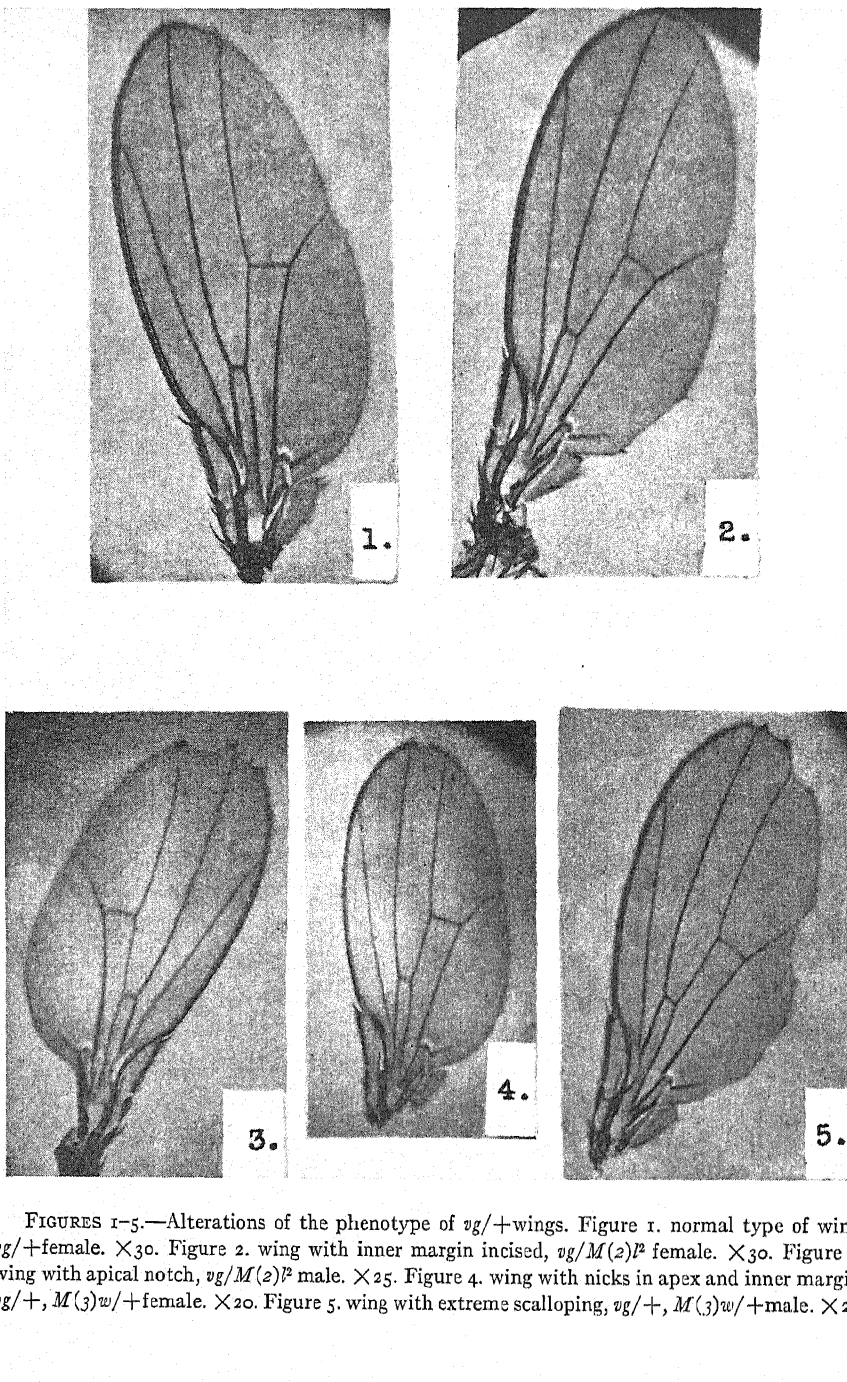
GENOTYPE	PERCENT WINGS+	PERCENT 1 WING SCALLOPED	PERCENT 2 WINGS SCALLOPED	TOTAL PERCENT SCALLOPED	N
♀ *vg*/+	100.00	0	0	0	257
♂ *vg*/+	99.60±1.27	0.40±1.27	0	0.40	248
♀ *vg*/+, $T1305D^+$	67.09±1.77	29.33±1.72	3.58±0.70	32.91	699
♀ *vg*/$M(2)l^2$	86.85±1.23	13.01±1.23	0.13±0.13	13.14	753
♂ *vg*/$M(2)l^2$	62.28±1.79	30.28±1.74	7.43±0.99	37.71	700
♀ *vg*/+, *M*(3)*w*/+	44.44±1.99	41.06±1.97	14.49±1.41	55.55	621
♂ *vg*/+, *M*(3)*w*/+	9.86±1.16	34.14±1.84	56.00±1.93	90.14	659

In table 1 the genotypes are arranged in sequence from the shortest to the longest length of life cycle. From the table it is apparent that the control counts are smaller than the counts in the several test groups. However they are in agreement with the control counts reported in our preliminary account (GREEN and OLIVER 1940) in which it was found that among the females *vg*/+ the frequency of wing scalloping was 0/365, while in the males *vg*/+ the frequency was 3/315. The preliminary data for control tests as well as those for the two Minutes were not included in this table because different culture media were used. The media of the earlier tests lacked brewers' yeast.

With each genotype tested, it is noteworthy that the males in addition to having a slightly longer developmental time also showed a greater frequency of wing scalloping than the females. An association between the developmental time and the frequency of wing scalloping is also observed with the various genotypes. On the basis of total wing scalloping (that is, the sum total of the flies with one wing incised and with two wings incised), the increase in scalloping is in accord with the increase in developmental time for a given genotype, with the one exception of the duplication *T1305* females. Although the prolongation of the life cycle was less in the $vg/+$, duplication *T1305* females than in the $vg/M(2)l^2$ females, the frequency of wing scalloping in the duplication females, 33 percent, more nearly approached that of the $vg/M(2)l^2$ males, 38 percent, and was greater than the frequency observed in the $vg/M(2)l^2$ females, 13 percent.

If the frequency of two wings scalloped is taken as a measure of the penetrance, it is apparent that the penetrance increased in the same order as the total wing scalloping frequency. In the controls no case of two wings scalloped was observed. This was true also of the controls of our earlier account (GREEN and OLIVER 1940). In the genotypes containing either the Minutes or the duplication, the least penetrance was observed in the $vg/M(2)l^2$ females, with only 1/753 individuals exhibiting two scalloped wings. The penetrance increased progressively with an increase in the length of the developmental period, until among the $vg/+$, $M(3)w/+$ males, 369/659 individuals possessed two scalloped wings. Again the data on the duplication females fail to fit the general results.

Because of the variability in the degree and region of the wing scalloping, no quantitative measurements of the reduction in wing size were attempted. However, certain variations in the general degree and region of the wing scalloping were noted among the genotypes tested. Among the $vg/M(2)l^2$ females, with the exception of one case, only one wing was affected. Generally, the nick or notch occurred in the inner margin of the wing in the region of the third posterior cell (figure 2). Occasionally the apical region, or the outer margin was affected. Among the $vg/M(2)l^2$ males, the frequency of two wings scalloped increased to approximately one-fifth of the total scalloped individuals. The degree of manifestation increased only slightly above that of the females of this combination. Generally, the apical region of the wing was nicked or notched (figure 3). Occasionally the inner margin was notched. The frequency of one wing scalloped among the $vg/+$, duplication *T1305* females was approximately equal to the frequency of this class among the $vg/M(2)l^2$ males. However, the frequency of two wings scalloped in the duplication females was considerably less than in the $M(2)l^2$ males and composed only about one-eighth of the total scalloping frequency of these females. With but few exceptions the nicking



FIGURES 1–5.—Alterations of the phenotype of $vg/+$wings. Figure 1. normal type of wing, $vg/+$female. ×30. Figure 2. wing with inner margin incised, $vg/M(2)l^2$ female. ×30. Figure 3. wing with apical notch, $vg/M(2)l^2$ male. ×25. Figure 4. wing with nicks in apex and inner margin, $vg/+, M(3)w/+$female. ×20. Figure 5. wing with extreme scalloping, $vg/+, M(3)w/+$male. ×25.

of the wings was confined to the apical region of the wing. Occasionally the wing was notched. Approximately one-fourth of the *vg*/+, *M*(*3*)*w*/+ females with scalloped wings had both wings scalloped. Either the inner margin of the wing was incised, or the apical region was nicked or notched. On a number of occasions both of the regions were affected in the same wing (figure 4). The most extreme manifestation occurred with the *vg*/+, *M*(*3*)*w*/+ males where almost two-thirds of the scalloped individuals had both wings scalloped. In almost every case the apical region of the wing was clearly notched, and in many cases the inner margin of the wing was incised at the same time (figure 5). In a few cases the inner and outer margins as well as the apex were incised, giving the wing the appearance of the Beadex phenotype.

DISCUSSION AND CONCLUSIONS

In the data presented it has been pointed out that a definite modification of the heterozygous vestigial wing phenotype results when either of two Minutes, $M(2)l^2$ and *M*(*3*)*w*, or a duplication of chromosome III, duplication *T1305*, is introduced.

The action of the Minutes on vestigial is probably general in nature, and associated in some manner with the prolongation of the developmental time. The Minutes are known to have a modifying effect upon various genes (DUNN and COYNE 1935; BRYSON 1940) other than vestigial. They do not cause scalloping of the wings; and therefore the increased frequency of scalloping in the combination of Minute with *vg*/+ is not likely to be an expression of the added individual actions of the two genotypes. However, duplication *T1305* may in part involve exaggeration, as will be discussed later. Although the Minutes cause a decrease in total growth (BREHME 1939), it does not seem possible to explain the scalloping on that basis. A decrease in body size occurs as a result of starvation of homozygous vestigial flies (CHILD 1939) and of crowding of Minute, *vg*/+ individuals (unpublished data); yet an increase in wing area occurs. If the general growth rate is a factor in scalloping, a further decrease in wing area would be expected with the smaller individuals.

Association with prolonged development

Each modifier, $M(2)l^2$, *M*(*3*)*w*, and duplication *T1305*, when introduced into *vg*/+ caused an increase in the length of the developmental time for the individuals as well as an increased frequency of wing scalloping. The similar direction of the prolongation of development and of the frequency of scalloping indicates that in some manner the two phenomena are associated in a causal way. With one exception, the genotype which caused an increase in the length of the life cycle caused a like increase in the frequency

of scalloping as measured by the total number of flies with scalloped wings. Similarly, the increased life cycle was associated with an increased degree of scalloping, as measured both by the quantity of wing present and by the number of flies with both wings incised. Individuals with the $M(3)w$ combination have a life cycle considerably longer than those with the $M(2)l^2$ combination, and the frequency and degree of scalloping are greater in the former than in the latter. Males have a longer life cycle than females; and with each combination, males have a higher frequency and to a lesser extent a greater degree of wing scalloping. However, the observed frequency of scalloping in the males exceeds the frequency expected if the effect is strictly proportional to the prolongation of the developmental time. A similar sexually dimorphic effect has been noted in temperature studies (HARNLY 1930; STANLEY 1931) and in starvation experiments (CHILD 1939) made upon homozygous vestigial.

The only discrepancy in the close similarity between the frequency of scalloping and the prolongation of development occurred with duplication *T1305*. On the basis of total length of life cycle, the frequency of scalloping in $vg/+$, *T1305* duplication females should be less than that observed in $vg/M(2)l^2$ females. The observed frequency, only slightly less than that for $vg/M(2)l^2$ males, was greater than expected. However, the prolongation associated with the Minutes occurs during the larval period (DUNN and MOSSIGE 1937; BREHME 1939). In our experiments, the length of life cycle for duplication *T1305*, as well as the Minutes, is a measure of the total life cycle. The exact stage associated with prolongation in the duplication is unknown. A second factor involved in the discrepancy may be the appearance of nicked wings in duplication individuals (2 percent in males) and in $vg/+$ males (0.4 percent). Since each genotype tends to produce scalloping, the combination $vg/+$, *T1305* duplication in the females might be expected to exaggerate the frequency of scalloping. It is known that the duplication in combination with Notch greatly exaggerates the Notch character (OLIVER 1937).

Although prolongation is apparently an important factor, the exact method by which it acts to increase the frequency and penetrance of vestigial is problematical. The gene for vestigial seems to be most susceptible to developmental modifications occurring during the larval period, and the Minutes prolong the larval period. Evidences for the larval susceptibility of the combination Minute, $vg/+$ have been shown by the response of the combination to temperature changes which were altered only during the larval period (GREEN and OLIVER 1940). The action of the prolongation upon the phenotype may be associated with the nature of the gene involved.

The gene for vestigial has been subjected to several studies in an at-

tempt to learn its nature of action. The data which we have presented do not clarify the picture but certain conditions are suggested.

If the gene for vestigial acts to produce a growth substance (GOLDSCHMIDT 1938) but does not produce enough of that substance for normal wings to differentiate, then our data on prolongation do not fit clearly into the hypothesis. Prolongation of development with the genetic combinations studied is associated with a decrease in wing area; and there is a definite correlation between length of the developmental period and the increased frequency of wing scalloping. The opposite effect should be expected if more time is allowed for vestigial to produce more growth substance.

If, however, the gene for vestigial acts to produce something which either inhibits or destroys a wing substance already present, prolongation as such might be a factor in scalloping. Delay in development during the sensitive period can permit the inhibiting substance to act for a greater interval of time, or permit the production of a greater amount of the substance. As a consequence, the wing which began as a normal structure would fail to develop completely. That type of action has been reported in a study of the pupal wings (GOLDSCHMIDT 1937), but no study of a similar nature has been made on the larval wing buds. That an inhibitory factor may be active during the larval period is suggested from the observations of CHEN (1929) who found that the wing anlage of wild-type larvae at pupation are larger than the vestigial anlage at the same period.

SUMMARY

1. A study of the effects of three genetic factors, $M(2)l^2$, $M(3)w$, and duplication *T1305*, all of which prolong developmental time, upon the wing phenotype in heterozygous vestigial was made.

2. It was found that in all cases studied the wing phenotype was modified in the form of notching and nicking of the wings. In each case the addition of the Minutes or the duplication significantly increased the frequency of wing scalloping over that of the controls.

3. The frequency and degree of wing scalloping were found to be associated with, but not proportional to the extent of prolongation of the developmental period by the Minutes; the greater the prolongation, the greater the frequency and penetrance of the wing scalloping. A sexually dimorphic effect was noted.

4. The frequency of wing scalloping which resulted with the introduction of the duplication was greater than that expected on the basis of prolongation of the developmental time alone.

5. It is suggested that the action of the Minutes is related to their effect on prolonging the action of the vestigial gene at a specific time during de-

velopment. It is also suggested that the action of the duplication is related to the prolongation of developmental time, as well as to the effect of the duplication alone on the wings.

6. The suggestion is offered that under the conditions of these experiments the gene for vestigial acts to elaborate some wing inhibitory or destructive substance.

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lines were isolated, one having the deficiency in the X chromosome and the other with a duplication in 3L. Cytological analysis indicates that nothing was lost in the transfer, that is, that all bands which are missing in the Notch region are inserted in the 3L heterochromatin. Genetic tests show that the deficiency involves the *w, rst, fa* and *dm* loci, while the inserted segment, when together with recessive alleles, shows spotting for *w, rst* and *fa* and the recessive mutant change in the *dm* locus. Neither the deficiency nor the duplication affects *pn* which is to the left of *w* nor *ec* which is to the right of *dm*, indicating that these two loci are outside the limits of the *N* 264-58 change.

The duplication is viable both in the males and in the females. Crosses were made between females heterozygous for different lethals in the Notch and white loci and males carrying the duplication to determine if it covered the lethal effect. In all cases where the lethal effect is covered hyperploid males should appear carrying the lethal in the X chromosome and the duplication in 3L. The following lethals were studied:

*N*264-32, a deficiency for four bands, 3C4 to 3C7 inclusive;

*w*258-45, a deficiency for a single band, 3C1;

*N*264-8, *N*264-40, *N*264-46, *N*264-47, all lethal Notches but without any cytologically detectable deficiencies;

*N*264-34, *N*264-53, *N*264-69, all translocations with one break adjacent to Notch and the other in the euchromatin of an autosome, none of them showing any detectable deficiency in the Notch region;

*N*264-62, a translocation with one break between 3C7 and 3C8 and the other in the heterochromatin of 2R, heterozygous with *w* and *fa*, shows mottling for both loci;

*N*264-48, an inversion with one break following 1B6 and the other between 3C7 and 3C8;

*N*264-52, an inversion with one break between 3C3 and 5 and the other between 20B1.2 and 20C1.2, showing spotting for *rst, fa, dm, ec* and *bi;* and

*N*264-63, a transposition of a piece from 13C to chromocenter into Notch region between 3C7 and 3C9.

The lethal effect was covered only in the case of *N*264-53. In that case *yN*264-53/In dl-49, *y Hw* m^2 g^4 females were crossed with *w* Dp264-58 males and the following F_1 offspring were obtained:

Females: 108

Males:	In dl-49, *y Hw* m^2 g^4	43
	y	13
	w	11
	w-mottled	12

In dl-49 are the normally expected males, *w*-mottled and *w* are non-dis-

junction males with or without the duplication respectively and *y* males have the Notch X chromosome covered by the duplication. While the wild-type/duplication males are fully fertile the *N*/Dp males obtained in this experiment are sterile. Moreover under normal conditions one would expect the *N*/Dp class to be one half of the dl-49, *y Hw* m^2 g^4 class while actually it is significantly smaller. This indicates that even in this instance the duplication did not entirely cover the lethal effect of *N*264-53.

Notch 264-85.—*N*264-85 was found on April 26, 1939 among the offspring of a cross between *y sc w* females and X-rayed wild-type Swedish-b males. The original female had notched wings and white eyes with red spots. Cytological analysis made by SUTTON (1940) shows a complex translocation in which an euchromatic section of X from 3C1 to 6A1.2 inclusive is inserted in its normal order in the chromocenter of the fourth chromosome between 101F and 102A. In addition there is a reciprocal translocation between 2R and X. The distal segment of 2R from 60A5 to the tip is attached to X at 6B1.2 and the distal portion of X from 3B3.4 to the tip is attached to 2R at 60A3.4. The light doublet 6A3.4 is unaccounted for and it may be either deficient or it may be obscured in the preparations because of the distortion caused by the rearrangement. If this doublet were present in the segment inserted in the chromocenter it would be very difficult to find it.

The section of the X chromosome inserted into the heterochromatin of the fourth includes 145 bands. The inserted piece segregates freely from the remaining X chromosome and consequently females deficient (or hypoploid) for the segment are expected to occur as well as both females and males hyperploid for the segment. Hypoploid females were not found indicating that the deficiency for such a long sector in this region of the chromosome is a dominant lethal. Hyperploids were found both among females and among males. Hyperploid females are fertile while hyperploid males are sterile showing that this long duplication upsets the physiological balance in males to such an extent that sterility is produced. A few 264-85 males were obtained from cultures kept at 28°C. These males showed mottling for *w*, *cx*, *cv* and for rough eyes (presumably *rst*, *fa*, *rg* and *rux*).

Crosses were made between Notch females which were heterozygous for the insertion and males carrying recessive alleles in the loci present in the inserted segment. All Notch females from such crosses have one normal X chromosome carrying the mutant and another which is involved in the translocation. These females showed mottling for *w*, *rst*, *fa* and *dm* located in the distal end of the insertion and for *rg*, *cx*, *cv*, *rux* and *vs* in the proximal end. This mottling is due to instability of the loci involved. The loci of *ec*, *bi*, *peb* and *rg* in the central portion of the insertion were found to carry wild-type alleles.

Since the hyperploid males are sterile, experiments could not be made to determine whether or not the duplication covers the lethals located in the duplicated section of the chromosome.

Notch 264-86.—*N*264-86 was found on September 18, 1939 as a single Notch female among the offspring of a cross between a *y pn* female and an X-rayed wild-type Swedish-b male. Cytological analysis made by SUTTON (1940) shows that a section of the X chromosome from 3C7 to 3E5 inclusive is inserted in its normal order into heterochromatin of 4 at 101F. This section segregates freely from the remaining body of the X chromosome and thus a deficiency and a duplication may be isolated. The deficiency is viable in heterozygous females and the duplication is viable both in females and in males.

Cytogenetic study indicates that the inserted piece is one band longer than the deficiency, which can be accounted for by the assumption that the chromosome was split at the time of the breakage and that both strands did not break at the same level (DEMEREC and SUTTON 1940).

The inserted segment includes 17 bands. When heterozygous for *fa* and *dm* it shows mottling for both loci. Notches *N*264-94 and *N*264-97, neither of which shows any cytological deficiency, were tested with the duplication to determine if their lethal effect would be covered. The results of these tests were negative. Also *N*264-53, which was covered by *N*264-58 duplication was tested with the duplication 264-86. From the cross *y N*264-53/dl-49, *y Hw* m^2 g^4×*w spl* Dp264-86 following offspring were obtained:

females:		364
males:	dl-49, *y Hw* m^2 g^4	176
	w spl	41
	w spl/Dp	30

Since *y* males did not appear it is evident that this duplication does not cover the lethal effect of *N*264-53. The *w spl* and *w spl*/Dp classes are due to non-disjunction.

Notch 264-100.—*N*264-100 was found as a single female on December 13, 1939 among the offspring of a cross between *y sc w* females and X-rayed wild-type Swedish-b males. This female had white eyes with red spots. Cytological analysis made by SUTTON (1940) shows that a piece of the X chromosome from 3C1 to 4B3.4 inclusive is inserted into the chromocenter of 3 between 80C and 81F. This region is unmapped on BRIDGES' chart. On the available cytological figures it has not been possible to determine in which arm of the chromosome the insertion is located.

The inserted piece involves 45 bands. Notch females heterozygous for *w*, *rst*, *fa*, *dm* and *ec* show mottling in all these loci. Genetic tests indicate that hypoploid females are viable and fertile. Hyperploid males have not

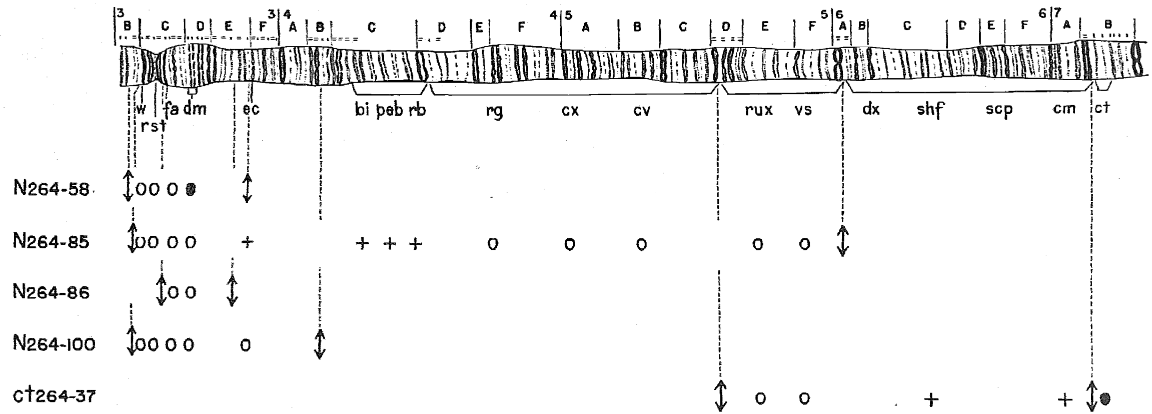

FIGURE 1.—Section of the BRIDGES (1938a) salivary chromosome map on which the position of loci is indicated. Each short line above the map represents a band and two horizontal lines indicate a doublet. The limits of each of the five insertions are shown by arrows, o's stand for loci which show mottling, full ●'s for loci in which recessive stable change has occurred and crosses indicate loci with wild-type alleles. (Last entry in the first column should read ct 268–37 instead of ct 264–37.)

been found among a large number of flies examined in the various tests. Since these tests include crosses with a number of different stocks as well as cultures raised at low and at high temperatures the non-appearance of males suggests that males carrying the duplication are not viable.

Cut 268-37.—*ct*268-37 was found on November 7, 1939 as a single *ct* female among the offspring from a cross between ct^n *g* females and X-rayed *y* males. Breeding tests showed that this change in the cut locus was connected with a lethal effect. This may be due either to a lethal allele of *ct* or, since this is an insertional translocation, the lethal may have been induced in connection with any one of the three breaks. Cytological study made by SUTTON (1940) shows that a piece of the X chromosome from 5D3.4 to 7B1.2 inclusive is inserted in an inverted position in the heterochromatin of 3L between 40F and 41A. In the inserted piece all bands which are missing in the X chromosome are accounted for. The insertion includes a section of 76 bands.

Hyperploid females and males and heterozygous hypoploid females are viable and fertile. Hypoploid females show a strong minute bristle character and have a lower viability. Genetic tests show that *rg*, *cx* and *cv* which are on the left side of the rearrangement and *sn* which is on the right side are not affected and thus are not located in the inserted segment. It has been already mentioned that *ct* shows a recessive change. It is known that the cut locus is represented by bands 7B3-5 (DEMEREC and HOOVER 1936) and since one of the breaks occurred adjacent to and to the left of 7B3 the locus *ct* is not located in the inserted segment. Tests with *rux* and *vs* show that recessive changes producing mottling have occurred in these loci. Tests with *shf* and *cm* indicate that the insertion carries wild-type alleles at these loci.

DISCUSSION

Experimental evidence presented in the foregoing pages is summarized graphically in figure 1. That figure shows the pertinent section of the salivary map of the X chromosome which has been copied from the revised Bridges' map (BRIDGES 1938a). The position of loci is shown as determined by work in this laboratory which is still unpublished.

An extensive study of mottling in the loci of the white-Notch region is now under way, and the results will soon be ready for publication. In the course of this study mottling has been observed in the following loci: *pn*, *w*, *rst*, *fa*, *dm*, *ec*, *bi*, *rg*, *cx*, *cv*, *rux* and *vs*. Results indicate that mottling occurs in conjunction with a chromosomal rearrangement when a certain locus is brought into the proximity of a specific region of a chromosome. As has been shown by SCHULTZ (1936) and later confirmed by a number of investigators heterochromatin is effective in inducing mottling. My studies indicate (Demerec in press) that not all regions of the heterochromatin

are equally potent, and also that certain sections of the heterochromatin do not induce mottling at all. On the other hand these studies suggest that all autonomous loci might show mottling given a suitable environment.

The diagrams in figure 1 demonstrate that all loci included in the insertions *N*264-86 and *N*264-100 show mottling. In the case of *N*264-58 all loci included in the insertion are affected, but only *w*, *rst* and *fa* exhibit mottling while *dm* shows a recessive mutant change. Such recessive changes are frequently observed in chromosomal rearrangements involving euchromatic segments. As a rule the locus which happens to be adjacent to the break is affected, and only in exceptional cases is the locus changed which is separated from the break by several bands. The length of the sensitive region within which a break may induce a change varies with the locus. A majority of the loci studied have a very short sensitive region.

The available evidence indicates that the sensitive region of the *dm* locus is of medium size and therefore the right break of *N*264-58 may well be within it and may be responsible for the recessive change in *dm*. In that case since the insertion is inverted the region of the heterochromatin of 3L between 80C and the centromere is effective in inducing mottling while the region to the left of 80C is not effective. However, in salivary gland chromosomes that region has the characteristic appearance of heterochromatin. A similar situation is found in *ct*268-37 where a section of the X chromosome is inserted in an inverted position into heterochromatin of 3L to the right of 80C in approximately the same position as in *N*264-58. Here again the region close to the centromere induces mottling while the region to the left of 80C does not. Since the cytological evidence indicates that the *ct* locus is not included in the inserted segment, the recessive change in *ct* is probably caused by a break in its proximity and a subsequent fusion to euchromatin.

A good illustration of the extent of the influence of heterochromatin is found in *N*264-85. Here a long segment of 145 bands is inserted in an inverted position in the heterochromatin of the fourth chromosome. Mottling is induced in the loci on each side of the inserted segment but the loci in the center are not affected. On the right side the effect stops between *rb* and *rg*. It is known that *rg* is located in the section between the 22nd and 81st band from the right end and therefore the effect on the right side extends through at least 22 bands and may reach as far as 80 bands It appears probable that the effect on the right side extends through a longer distance than the effect on the left side. It is of interest to note that the right side is attached close to the centromere. On the left side the effect stops between *dm* and *ec*. Since *dm* is located in the 13th and 14th band from the left break and *ec* is the doublet represented by the 27th and 28th band from the break, the effect on the left side extends through at least 14 bands and it stops before the 27th band is reached.

Evidence is available showing that the effect in this region of the chromosome may spread to a longer section than 14 to 26 bands. In *N*264-52, which we have in our collection, a section from 3C4 to 20B1.2 inclusive is inverted and thus the *N* region is brought into the proximity of heterochromatin. In this case mottling is evident in *rst*, *fa*, *dm*, *ec* and *bi* and therefore the effect extends at least 50 bands from the break.

The material presented here indicates that certain regions of heterochromatin are not effective in inducing mottling. The data show that the region to the left of 80C was ineffective in both *N*264-58 and *ct*268-37. In these cases that region is moved away from the centromere by insertions. It may be argued that the increase in the distance from the centromere may be responsible for the non-appearance of mottling. However, the inserted segment in *N*264-85 is much longer than either in *N*264-58 or *ct*268-37 and in spite of that the heterochromatin is effective in producing mottling. I am inclined to think that the quality of the heterochromatic region as well as its relation to the centromere and its quantity are all important factors in determining mottling. The evidence in support of this view was discussed in a paper read at the Seventh International Genetics Congress in Edinburgh (DEMEREC in press).

Changes which occurred in the inserted segments are best visualized by analyzing biological effects produced by them. The most striking and at the same time the most characteristic effect is mottling. As an illustration I will use the mottling observed at the white locus. In all cases mentioned in this paper the females heterozygous for the insertion and the white gene have eyes prevailingly cream or very light cherry with smaller or larger spots colored dark cherry or red (wild type). Judging from the appearance and the distribution of spots it seems probable that red spots originate through changes from light color into dark color which have occurred during the development of the eye. If this is expressed in terms of genes it may be said that in light regions the action of the wild-type allele of the white gene, which was present in the segment before insertion, is partially or totally suppressed. Total suppression produces white background and partial suppression light color. In red spots the activity of the gene is again fully restored. It is important to note that in the case of large red spots all facets in a spot are red, indicating that when the restoration of activity occurs it persists among the daughter cells.

The question now arises concerning the mechanism involved in the suppression and the restoration of the activity of the affected genes. Two possibilities are evident. (1) We may be dealing here with real chemical changes in genes which are unstable and revert to the original state. In this case it would be assumed that the change in the position of the locus produced a reversible chemical change in the gene and that reversions occur during the ontogeny of the fly whenever conditions are suitable. (2) It is also possible

that because of the shift in position the action of the gene is suppressed without any change in the chemical constitution of the gene itself. In such a case also certain physiological conditions arising during the ontogeny of the fly would reestablish the activity of the gene and thus bring about the development of wild-type spots. I intend to discuss the evidence in favor and against these two possibilities in another paper where experiments dealing with 35 cases of mottling will be described. In this paper I am only considering the facts which are specific to the material described here.

From a theoretical standpoint the experiments in which the covering effect of duplications was tested are of particular interest. Tests with duplication *N*264-58 showed that of 12 lethals connected with the Notch phenotype only one, namely *N*264-53, was covered by the duplication and in addition one lethal white tested was not covered. One of the Notches and the white were cytologically detectable deficiencies while all other Notches had a full complement of bands in salivary chromosomes. Similarly neither of the three lethal Notches tested which included also *N*264-53 was covered by *N*264-86 duplication. These results show (1) that these duplicated segments inserted in the heterochromatin were not only unable to cover a physical deficiency for a small part of the duplication but in the majority of cases they were unable to cover a biological deficiency which is expressed as a lethal; (2) that there is a difference between the Notches in regard to lethality and (3) that there is a difference between similar duplications in their ability to cover the lethal effect of loci which are present in duplication.

The inserted segment of *N*264-58 shows mottling for *w* and for *fa*, which is considered an allele of *N*, but was not able to cover a deficiency affecting either of these two loci. It has been shown by POULSON (1940) that the abnormalities in development which bring about the death of the hemizygous flies are evident in very early embryonic development, during blastoderm formation, in the case of Notches and somewhat later in the case of white lethals. This indicates that in early embryonic stages the function of white and facet-Notch genes in the inserted segment of *N*264-58 is not adequate to cover deficiencies in these loci. However, the wild-type spots which appear on the eyes of flies indicate that their function is normal in some of the cells during late stages of development.

Another interesting fact brought out in these experiments is the difference in viability of duplications. It has been shown that males carrying the *N*264-85 duplication are viable although they are sterile, while the males carrying the *N*264-100 duplication have never been found and presumably this combination is lethal. Both duplications have identical left limits. The *N*264-100 segment includes 45 bands and the *N*264-85 segment includes in addition to these same bands 100 others. It is evident that the lethal effect

of *N*264-100 cannot be due to the unbalancing effect of the total number of genes present in the duplicated segment, since the total number of genes in N264-85 duplication is much larger. The lethal effect could be brought about only through the unbalance of the genic system caused by the activity of the genes located in the duplicated segment, which activity is determined by the position of the segment in the gene system.

SUMMARY

Description is given of five insertional translocations in which euchromatic pieces of the X chromosome are inserted into heterochromatin. Figure 1 summarizes the results of cytogenetic analysis.

As a rule the loci brought into the proximity of heterochromatin show mottling. When the inserted segment is long, as in the case of *N*264-85 where it includes 145 bands, the loci on both ends of the segment show mottling while the loci in the center are not affected.

In *N*264-58 and *ct*268-37 segments are inserted in 3L to the right of 80C in an inverted position, and in both cases the loci farthest from the centromere do not show mottling.

A number of Notches and one lethal white were tested with duplications *N*264-58 and *N*264-86. In all but one Notch the duplication did not cover the lethal effect.

The inserted segment in *N*264-85 includes all bands of the segment in *N*264-100 and in addition 100 bands more. In males the duplication for *N*264-100 is lethal while the duplication for *N*264-85 is not, indicating that in this case the length of the segment is not responsible for the lethal effect.

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TERMINAL DEFICIENCIES IN THE X CHROMOSOME OF DROSOPHILA MELANOGASTER

EILEEN SUTTON
Carnegie Institution of Washington, Cold Spring Harbor, New York

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IN THE course of cytogenetic studies on the X chromosome in *Drosophila melanogaster* conducted in this laboratory by DR. M. DEMEREC, several terminal deficiencies have been found.

Three of these have already been described by DEMEREC and HOOVER (1936) and it is the purpose of this paper to describe others and to put forward evidence that they are really terminal deficiencies in which a broken chromosome has failed to rejoin either in the old or in a new combination, so that a new chromosome end is formed at the breakage point.

The standard nomenclature for genes and chromosomal changes in Drosophila (as in Drosophila Information Service No. 10) will be used throughout as follows: genes in the X chromosome with positions on genetic map—*ac*, achaete (0.0+); bb^l, bobbed lethal (66.0); *car*, carnation (62.5); *f*, forked (56.7); *Hw*, hairy wing (0.0+) *l J1*, lethal J1 (−0.0); *N*, Notch (3.0); *pr*, prune (0.8); *sc*, scute (0.0+); *spl*, split (2.9±); *w*, white (1.5); *y*, yellow (0.0).

genes in other chromosomes—

2nd chromosome: *bw*, brown; 3rd chromosome: *e*, ebony; 4th chromosome: *ey*, eyeless; *M-4*, Minute-4; sv^n, shaven-naked.

chromosomal changes—

Df, deficiency; Dp, duplication; T, translocation

METHODS

Slides of the salivary gland chromosomes of female larvae heterozygous for the different changes were prepared by the acetocarmine technique.

The equipment used in analysing the slides consisted of a 90 × 1.3 N.A. apochromatic objective, an oil-immersed 1·4 N.A. condenser, 12.5 × compensating oculars, and a Bausch and Lomb research lamp with the green Wratton filter number 61.

Cytological analyses were based throughout on BRIDGES' (1935 and 1938) maps of the salivary chromosomes.

ANALYSIS OF THE CHANGES

1 Df(1) 260.10.

This deficiency came from a mating of *y sc w* females with wild Swedish-b males which had been treated with X-rays. It was identified in an F_1 female which was phenotypically yellow, indicating that the *y* locus of the paternal

X chromosome was changed or deficient. The *ac* locus was found to be affected also.

Females carrying the mutant X chromosome (*y ac*) over normal *y sc w* were found to be heterozygous for a deficiency involving bands 1A1 and 2 of the salivary chromosomes. The appearance of the figures suggests that the whole tip to the left of 1A3 has been lost.

Males with the deficient X are viable and fertile, and must therefore carry the normal allele of the *lJ1* locus, which, according to MULLER, lies to the left of the *y* locus. Similarly, Df(1) 260.5 (DEMEREC and HOOVER 1936) has four bands missing, but is perfectly viable and fertile.

Data obtained in this laboratory show that the genes *y* and *ac* must both be located in the region 1A 5–8 and that their change was in this case due to "position effect." The work of MULLER (1935), and cytological results of MULLER, PROKOFYEVA and RAFFEL (1935) do not exclude the possibility that *lJ1* also is located in this region. This would allow deficiencies such as these to lack the extreme tip of the X chromosome and yet to carry the normal allele of the *lJ1* locus.

2. Df(1) 260.19.

This deficiency was picked up from cytological observations on a stock of STERN ($\widehat{yy}/g^2$ *B*) and must have occurred spontaneously. It resembles 260.10, cytologically, the break being in the same position between 1A2 and 3. It differs genetically as the loci of *y* and *ac* are unaffected. The males are viable and fertile.

3. Df(1) 260.25.

Genetical data. The stock carrying this deficiency was derived from an F_1 female carrying a normal *y sc w* chromosome and an X chromosome from an irradiated Swedish-b male. This female was phenotypically scute, and when mated with *y sc w* males she gave the following offspring:

	Females	Males
sc	11	—
y sc w	15	13
y sc	13	—
sc w	6	8

This progeny shows three peculiar features: (1) There is apparently an abnormally high crossover value for the *y-sc* and/or *sc-w* regions, giving relatively large numbers of *y sc* and *sc w* flies. (2) The *y sc* type is inviable in the hemizygous condition (no *y sc* males). (3) The mutant *sc* type is not recovered in the hemizygous condition (no *sc* males).

These results could be accounted for on the supposition that the tip of X including the y^+ gene had been translocated to some other chromosome region where it segregated with comparative freedom from *sc* and *w*, while

the break at the tip of X had caused a mutation in the *sc* locus accompanied by a recessive lethal change. The *sc* and *y sc w* classes would then represent the parental types (*sc* failing to appear among the males on account of the lethal). The *y sc* flies would be heterozygous for a deficiency including the *y* locus, this deficiency probably being lethal in males, which would in any case fail to survive on account of the lethal *sc* mutation. The *sc w* class would carry two normal *y sc w* chromosomes together with a duplication for the tip region including the y^+ gene.

On closer examination the *sc* and *sc w* flies showed a variegated body and bristle color, predominantly wild-type with patches of yellow, suggesting that the y^+ gene was inserted in some chromocentral region. Mosaicism of wild-type and mutant tissue is characteristic of such changes (SCHULTZ 1936).

A test mating with *y ac* showed that variegation of the achaete character also occurred. The break in the X was to the right of *y* and *ac*, therefore, but probably to the left of *sc*, because it showed no variegation.

In order to locate the translocated segment carrying y^+ and ac^+, a cross was made between females of a stock, ($\widehat{yvf}$; *bw*; *e*; *ey*) and *sc w* males assumed to carry a normal *y sc w* chromosome and a duplication for y^+ ac^+. The F_1 consisted of 309 *y v f* females and two wild-type females with 369 *sc w* males and one *y v f* male. The F_1 females receive both their X chromosomes (attached) from their mother, while the males receive their X chromosome from the father; the other chromosomes are distributed at random. Since all females (with two exceptions) were yellow and all males (with one exception) were wild-type with respect to the yellow locus, the duplication clearly segregates with the X chromosome. The exceptions could be accounted for by the rare formation of detached X's by crossing over in the female parent, whereby one son received a *y v f* chromosome from the mother, and two daughters received only one *y v f* chromosome from their mother, the second X chromosome with the y^+ duplication being derived from the father. These data showed that the tip region of the X was transposed to the chromocenter of the same chromosome.

A linkage test was made with genes at the proximal end of the X chromosome to determine the position of the duplication in respect to these genes. The back cross *y* Dp/*y f car* $bb^l \times$ *y f car* bb^l gave the results shown in table 1. In this experiment the flies were raised at 25°C in order to get crossover values comparable with the standard values.

From this mating all classes of females homozygous for bb^l are lethal and thus lacking. Crossing over between *f*, *car*, and Dp is therefore shown adequately in the males only. The crossover value between carnation and the duplication calculated from the males is 3.41 ± 0.40 percent, so that it is not detectably different from the crossover value for *car*-bb^l (3.5 percent).

Crossing over between the duplication and bb^l could be detected only in the females. Since double crossovers between *car* and bb^l would not be expected, the single yellow among these shows that the duplication is genetically to the right of, and very close to, bb^l, giving less than 0.1 percent of crossing over.

A cross was made between females carrying the deficient X chromosome and a normal X marked by the dominant gene *Hw* and males of the *lJ1 sc J1* stock in which the lethal change is covered by a deleted X chromosome. From this cross (Df/*Hw*×*lJ1 sc J1*—del 24), half of the F_1 females should have the deficient X chromosome and a *lJ1 sc J1* chromosome,

TABLE 1

Offspring of backcross y Dp/y f car bb^l×*y f car* bb^l.

FEMALES			MALES		
non-crossovers	Dp	1184	non-crossovers	Dp	1002
	y f car bb^l	—		*y f car*	815
region 1	*f* Dp	36	region 1	*f* Dp	69
(*f-car*)	*y car* bb^l	—	(*f-car*)	*y car*	43
region 2	*f car* Dp	35	region 2	*f car* Dp	33
(*car*-bb^l)	*y* bb^l	—	(*car*-Dp)	*y*	32
region 3	*y*	1	regions 1, 2	*y f*	—
(bb^l-Dp)	*f car* bb^l Dp	—		*car* Dp	3
Total		**1256**	Total		**1997**

and only half of these should carry the covering deletion. If the deficiency includes the normal allele of *lJ1*, the *sc* flies which lack the deletion will be inviable, and the ratio of *Hw* to *sc* in the F_1 females will exceed 1:1. If the normal allele of *lJ1* is still present in the deficient chromosome, all *sc* females should be viable whether they carry the deletion or not, and the ratio of *Hw* to *sc* should be 1:1.

A very great excess of *Hw* females was actually found, the total numbers being 171 *Hw*:17 *sc*. It is evident that there is some factor disturbing the ratios other than the failure of the *sc* females which lack the deletion, and the test is therefore rather unsatisfactory. It is not apparent, however, that the normal allele of the *lJ1* locus is still present in the deficient chromosome.

On the genetical evidence, the deficiency may include all genes to the left of *sc*, and may therefore be a deficiency for the entire tip of the X chromosome.

Cytological data. Female larvae heterozygous for the *sc* change were obtained from the mating *sc/y sc w*×*y sc w*. Their salivary gland chromosomes showed an abnormality at the tip of X, where all bands to the left

of 1B 3.4 were missing in one of the strands. A further peculiarity was the presence of a nucleolus-like structure at the deficient tip, which looked as if material had oozed out from the end of the chromosome (Plate 1, fig. C). This phenomenon is common as a casual occurrence in figures where a partial or complete break has occurred (not induced by irradiation) in one of the "weak spots" of the chromosomes (for instance, in region 11A); but in this case it appeared consistently in every figure. This is regarded as evidence that the newly-constituted chromosome tip consists of the naked broken end, without any other material attached to it.

It proved impossible to locate the translocated segment 1A1 – 1B1.2 at the X chromocenter, so it is not known whether it is inserted in an intercalary position or attached terminally to the short arm.

It was considered desirable to test the capacity of the new end for rejoining with other broken ends, and for this purpose viable males carrying the tip deficiency were obtained by mating the *y sc* females (heterozygous for the deficiency) with Dp(1) 118, thus introducing a fragment, made up of the extreme distal and proximal regions of the X, which covered the lethal effect of the scute change and of the deficiency.

Males carrying the 260.25 deficiency and Dp 118 were treated with X-rays at a dosage of 4000r, and mated to wild Canton-S females. Slides were made from F_1 female larvae, which had both a normal X and the irradiated deficient X.

In 102 pairs of glands, 27 aberrations were obtained with a total of 63 breaks, but in no case had the broken tip of the deficient X become attached to one of the newly broken ends. It is apparent, therefore, that healing has taken place, so that the tip does not tend to rejoin as is usual within a short time after breakage has occurred.

4. T(1; 4) 258.53.

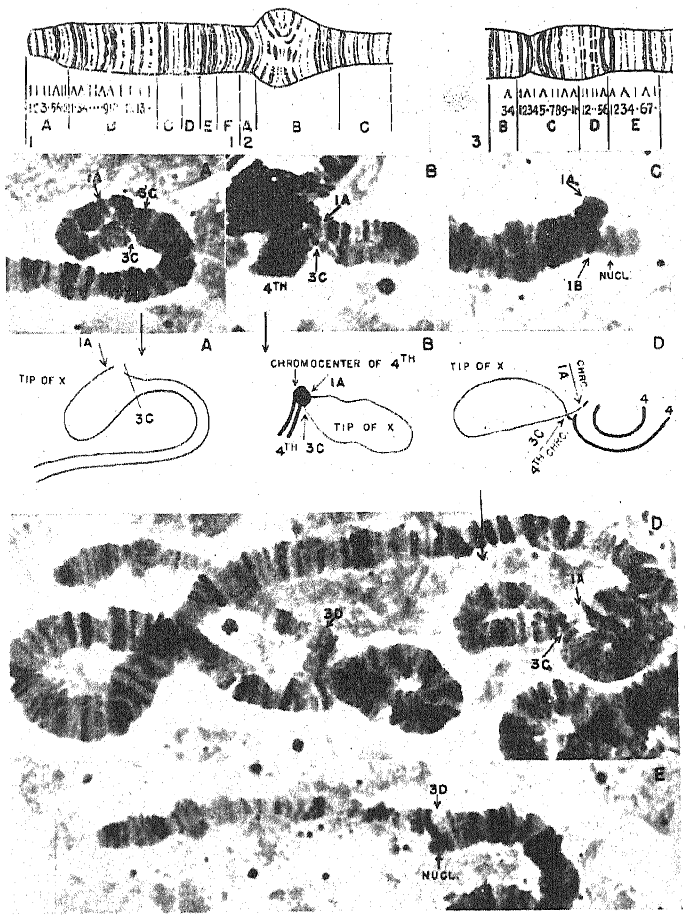
The existence of eye color variegation in an F_1 female from the mating of *y sc w* by X-rayed Swedish-b male indicated a translocation of the white locus (band 3C1 in the salivaries) to the chromocenter.

Observation of the salivary chromosomes showed that the tip of the X

LEGEND FOR PLATE 1

Top: Diagrams of regions of X chromosome from BRIDGES' salivary chromosome map.

Below: A. T(1; 4) 258.53, haploid tip of normal X bent back so that distal region (1A) pairs with distal end (3C) of deficient X (see diagram). B. T(1; 4) 258.53, tip of X attached to fourth chromocenter, distal end (1A) also pairing with chromocenter (see diagram). C. Df(1) 260.25 lower strand, deficient for 8 distal bands, shows nucleolus-like structure extending beyond normal tip of upper strand. D. T(1; 4) 264.113 at left, haploid tip of normal X and deficient strand ending at 3D; at right, tip of X attached to 4th chromocenter, distal end also bent back to pair with chromocenter (see diagram). E. T(1; 4) 264.113, haploid tip of normal X and, above, deficient strand ending in a small nucleolus-like structure.


A
B
C
D
E
F
1
2
3
1A
3C
4TH
1B
NUCL.
TIP OF X
CHROMOCENTER OF 4TH
4
3D

up to and including 3C1 was attached to the chromocenter, apparently at the base of the fourth chromosome, but there seemed to be two normal fourth chromosomes, and there was no foreign material attached to X distal to 3C2.3 (Plate 1, figs. A, B).

The salivary configurations suggested two possibilities: (1) that the tip of X was attached to the short arm of the fourth, in which case it was possible that a piece of this arm, so small as to be practically invisible, had been translocated to the distal end of the X; or (2) that the tip of the X had been attached to the long arm of the fourth, near the proximal end, in which case the broken distal end of 4R must have been lost, the broken tip of the X remained unattached at 3C2.3, and non-disjunction of the fourth chromosome must have occurred subsequently to restore the second normal fourth chromosome.

A cross with the fourth chromosome recessive sv^n decided between these alternatives. The F_1 from the mating Df$+w^{mot}-4/y$ *Hw w*; $+^{sv}/+^{sv}\times w$; *sv* gave the following classes:

$w^{mot}+$	27
w^{mot} *sv*	13
$w+$	32

The presence of *sv* flies in the F_1 in the class which also carried the X-4 chromosome responsible for white mottling, can be explained only by the second alternative. The break in the fourth chromosome must have been proximal to the *sv* locus, so that the X-4 chromosome lacks the wild-type allele of this locus. The three fourth chromosome centromeres apparently segregate more or less at random, and some of the gametes carry no normal fourth, but only the X-4 chromosome, deficient for the *sv* locus. These gametes are responsible for the w^{mot} *sv* flies appearing in F_1 from the mating with the *sv* stock. These flies must be haploid for all of the fourth chromosome except the proximal region.

The subsequent discovery of Minute flies in the T(1; 4) 258.53 stock supported this explanation. These Minutes must be due to the same independent segregation of the X-4 chromosome from the two normal fourths, and they are comparable with BRIDGES' M-4, which is haploid for a part of the fourth chromosome owing to a deficiency (BRIDGES 1935).

In view of this evidence, the end of the abnormal X, broken to the left of 3C2.3, can be considered as having reconstituted itself as a normal end without the attachment of any previously existing end structure.

5. T(1; 4) 264.113.

This aberration was obtained in a Notch F_1 female from a mating of *y pn* females with irradiated Swedish-b males. It closely resembled the previous one, 258.53 (Plate 1, fig. D) but differed cytologically in two respects:

(1) the break in X occurred between 3C9·10 and 3D1; (2) the unattached broken end, terminating to the left of 3D1, frequently showed a nucleolar structure, similar to that found in Df(1) 260.25, except in that it was much smaller. This exudation sometimes obscured the distal bands of 3D (Plate 1, fig. E).

The cytological difference in the position of the break corresponded with a genetical difference. This stock showed a Notch mutation and mottling of *w* and *spl*, all of these loci being transferred to the fourth chromocenter, whereas in 258.53 only the *w* locus was affected.

DISCUSSION

The special behavior of chromosome ends, namely the fact that they do not as a rule become attached permanently to one another or to newly broken ends, but persist as ends, has led to a supposition that they must have a special structure which conditions their behavior. The name of telomere has been applied to this terminal structure, and it has been supposed that reproduction of the telomere maintains the identity of the chromosome end.

The literature provides occasional examples of behavior which is inconsistent with this view of the chromosome ends. Thus, although STADLER (1932) remarked on the absence of terminal inversions in maize, such an inversion has subsequently been found in *Drosophila ananassae* (KAUFMANN 1936). A further example of anomalous behavior of chromosome ends has been found by MCCLINTOCK (1939), who described chromosomes derived from the breaking of dicentric anaphase bridges during meiosis in maize. These chromosomes, though not deficient, have broken ends. In the endosperm the broken ends of half chromatids undergo a repeated cycle of fusion, dicentric bridge formation, and subsequent rupture. In the sporophytic tissue, however, no anaphase bridges are found. It is apparent that one of the chromosome set in this tissue was derived from breakage of a dicentric bridge, because of the absence of a characteristic knob; and it is therefore evident that the broken end has healed so as to form a normal, permanent chromosome end.

MULLER (1938) has questioned the existence of genuine terminal deficiencies in Drosophila, on the grounds that chromosomes with such deficiencies would lack a stabilizing telomere structure.

The simplest explanation of the chromosome ends described in this paper is, however, that they originated by loss of the whole distal portion of the X chromosome, and that the broken ends healed and became functionally normal. There is no evidence to contradict this explanation. On this view, stable ends can be formed de novo from broken ends without attachment of a previously existing telomere. There seems no reason to doubt this

interpretation of these and other apparent terminal deficiencies in Drosophila, as well as the results of McCLINTOCK in maize.

SUMMARY

Five apparent terminal deficiencies in the chromosomes of *D. melanogaster* are described. From genetical and cytological data it is concluded that these are genuine terminal deficiencies, in which the normal chromosome end has been lost and a new end has been formed at the breakage point. This conclusion is incompatible with the assumption that existing chromosome ends, or "telomeres," are essentially permanent and indispensable structures.

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ANALYSIS OF WILD AMERICAN RACES OF OENOTHERA (ONAGRA)[1]

RALPH E. CLELAND
Indiana University, Bloomington, Indiana

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THERE was a time when the evening primrose was the despair of the geneticist. It still is the despair of the taxonomist, who finds it difficult to classify and delimit species in this genus on the basis of phenotypic criteria alone. This difficulty is now known to be due to the fact that most Onagras are, from the phylogenetic point of view, not single, but dual entities. They are usually composites of two distinct, separable, and, as a rule, phylogenetically diverse components or genoms known as the RENNER complexes, one of which may largely dominate over the other phenotypically. These complexes are continuing entities of a more or less permanent nature, being passed intact from generation to generation in selfed lines.

The peculiar cytogenetic behavior of Onagra, which is responsible for this duality, is now familiar to all geneticists and will not be discussed here. The fact should be emphasized, however, that the genoms which are associated to form a given species are, in most races, very different from each other genetically, and probably of diverse phylogenetic origin. Thus, in tracing the affinities of a given race, one must consider two distinct lines of phylogenetic descent. The relationships of both genoms must be taken into consideration.

This is not an easy thing for the taxonomist to do, however, since one genom, by reason of its dominant genes, often effectively masks the other. Furthermore, races composed of two diverse genoms breed as true as do those whose genoms are essentially similar, thanks to their balanced lethals. Hence a taxonomist cannot tell by observation, or even by inbreeding, which races contain genoms of diverse phylogenetic origin and which contain genoms which are closely related. This can only be determined by cytogenetic means.

One example will show the necessity of the cytogenetic approach in tracing the phylogenetic position of a race in Onagra. "*Iowa 1*" is a race of the *biennis* type. It has broad, dark green crinkly leaves, small flowers and other so-called "*biennis*" characters, and it breeds true to type. So far as one can see phenotypically, it is *biennis* and nothing else. If one crosses it, as egg parent, to the *hookeri* of DEVRIES, the F_1 hybrids resemble "*Iowa 1*" very closely. But if one crosses it as pollen parent to *hookeri*, a very different sort of hybrid results, one which looks in no respect like a *biennis*, which differs from its reciprocal in almost every external character. Both

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hybrids have received the same genom from the homozygous *hookeri;* the differences between the two must therefore be due solely to "*Iowa 1*" itself, which, it turns out, carries a genom very different from the one which is able, by reason of numerous dominant genes, to control the phenotype. One would never have suspected, on looking at the *biennis* type, "*Iowa 1*," that it was carrying a genom capable of producing a plant so different from itself. Yet it is obvious that it is just as important to consider the affinities of this complex as of the other in determining the phylogenetic position of "*Iowa 1*."

This is not an isolated case. It is typical of the genus as a whole. What is true of "*Iowa 1*" is true of most of the wild races east of the Rocky Mountains. It at once becomes apparent, therefore, that a proper understanding of relationships in Onagra can be reached only through the determination of relationships between genoms. If we understand the phylogenetic position of the genoms of a given race, then we can understand the position of the race itself. This can, however, only be accomplished by cytogenetic means, for only by such means can one ascertain, first, whether a race is made up of two diverse genoms, and secondly what are the relationships between the genoms found in the various wild races.

We may now ask how it is possible, by cytogenetic means, to determine relationships between various genoms. This can be done to some extent by analyzing each genom genetically and comparing the genoms thus analyzed, a type of study made noteworthy by the work of RENNER. It is obvious, however, that a method necessarily so slow cannot be used in an attempt to determine relationships throughout the entire genus. Another method, however, which promises to make such an analysis possible depends upon the fact that relatively frequent reciprocal translocations in Onagra have resulted in many diverse arrangements or groupings of the end segments of the chromosomes. Each genom has its own characteristic segmental arrangement. When two complexes with diverse arrangements of segments unite to form a plant, the pairing of homologous segments results in the formation of chains instead of pairs. By studying the degree to which a given complex produces chains or pairs with certain standard complexes whose segmental arrangements are known, it is possible to analyze the segmental arrangement of this complex in terms of these standards and to determine to what degree and in what manner this complex bears a resemblance to other complexes from the standpoint of segmental arrangement.

It has been suggested in earlier papers (1931, 1935, 1937) that similarity in segmental arrangement is likely to be an indication of close phylogenetic relationship. Complexes which have similar segmental arrangements are likely to be closely related. This gives us a simple and unique tool with

which to study relationships in Oenothera. One has but to cross the various races, determine which complexes have similar arrangements of segments, and thus obtain excellent clues as to their phylogenetic relationships. It may be added, however, that this method, which is capable of tracing to some extent the record of the interchanges which have occurred in the evolution of a complex, is not so capable of ascertaining the sources whence its genes have come. Without entering into a discussion of this matter at present, it may merely be said that it is possible for a complex to acquire genes in more than one way from an associated and unrelated complex without the alteration of its segmental arrangement.

For several years the writer has been working, with the collaboration of DR. P. A. MUNZ, of Pomona College, upon an analysis of the relationships between the various wild races of Oenothera. This work has been supported for five years by the American Philosophical Society and is now being assisted by the Rockefeller Foundation. It is our plan and hope to make first a broad survey in order to gain a picture of the general situation, after which more detailed studies of individual groups of races and of geographical areas will serve to make clear the details. As yet, we have gained only a rough idea of the situation, but we have gone far enough to feel certain that our method, if pursued far enough, is capable of yielding a clearer and more precise picture of evolutionary trends and relationships than has been hitherto obtained in this and perhaps in any genus.

We have, so far, grown and determined the chromosome configuration of about 140 races (not counting European races) and have studied hybrids involving 96 of these. In the case of some races one or both complexes have been completely analyzed; in the case of other races only a bare beginning has been made or the analysis is in one stage or another of completion. Out of this mass of information obtained from our crosses, details of which will be presented elsewhere, we are beginning to understand the relationships of some races or groups of races, but of others we know as yet little or nothing.

The groups of races which give evidence of having distinctive cytogenetic behavior will now be mentioned.

The hookeri alliance. This group of races is centered chiefly in the far southwestern portion of the United States and in northern Mexico. In all, 36 races have been examined. The group is characterized by large flowers, open pollination, paired chromosomes or small circles in diakinesis, and the absence of lethal factors. The commonest condition is the all-pairing condition; circles of four are not infrequent, but larger circles are rare. Circles involving more than eight chromosomes are unknown. The presence of numerous pairs or of small circles means, on the basis of our assumption, that the genoms making up any individual race of the *hookeri* type are

closely related to each other in segmental arrangement, and probably, therefore, are phylogenetically closely related.

When various races of *hookeri* are crossed, the hybrids between them also show mostly paired chromosomes or circles of four. Larger circles have rarely been observed. It is clear from this that genoms in different *hookeri* races are as closely related on the average as are genoms within individual races. In general, the conclusion seems unavoidable that the *hookeri* alliance is made up of a variety of races, all of which, however, are closely related to each other, as is indicated by the fact that their genoms are similar or identical in segmental arrangement.

Of the various segmental arrangements present in the races of *hookeri* which we have studied, one is much more commonly found than any of the others. This arrangement is written, in terms of the originally chosen standard (^{h}*hookeri* of deVries) 1·2 3·4 5·6 7·10 9·8 11·12 13·14. There is reason for considering this arrangement either ancestral to all others in Onagra or, at least, the oldest arrangement now extant in California.

The second group of races with distinctive cytogenetic behavior is the *irrigua* alliance. In New Mexico, and possibly in contiguous areas, a type exists known as *irrigua*. It resembles the *hookeris* in being large-flowered, open-pollinated, and alethal, but it possesses certain fairly distinctive phenotypic traits which have been supposed to set it more or less apart. I have examined some seven races of this type and find them to be composed for the most part of individuals having chromosome configurations[2] of intermediate character, such as a ⊙6 or a ⊙4, ⊙6 or a ⊙8. Because of the absence of lethals, however, these plants do not breed true to their configurations. A plant with a circle of four, a circle of six, for instance, will throw, in addition to the parental type, individuals with a circle of four alone, a circle of six alone, or seven pairs. Circles are maintained in the population, however, because open-pollination encourages crossing between individuals having genoms with different segmental arrangements.

When the *irriguas* are crossed with the *hookeris*, hybrids with intermediate configurations predominate, such as ⊙8, or a ⊙4, ⊙6. Since intermediate configurations such as these represent minimum differences between the complexes of, in most cases, two or three interchanges, the genoms of the *irriguas* give evidence of fairly close relationship both to the genoms of the *hookeris* and to those with which they are normally associated. In short, the *irriguas* have a variety of segmental arrangements which differ in moderate degree from each other and at the same time also differ in moderate degree from the *hookeri* arrangements.

The *irriguas* are of particular interest in the fact that they are almost the only races of Onagra so far found in the wild whose chromosome con-

[2] ⊙ signifies "circle of."

figurations are intermediate between the all-pairing condition and the presence of large circles. It is a curious fact that races with intermediate configurations are almost entirely confined (so far as our work shows) to New Mexico and adjacent areas. Certain strains of *longissima* from Utah, of *Jamesii* from Oklahoma, and of *hookeri* from New Mexico and Colorado have shared with *irrigua* this peculiarity. It is no doubt a significant fact that intermediate configurations should be found primarily in a region intermediate between the western area, in which the all-pairing condition predominates, and the eastern area, in which a circle of 14 is the all but universal configuration. It looks as though these forms may represent transitional stages in the development of one condition (probably the eastern) from the other (probably the western).

We come now to a third group possessing distinctive cytogenetic behavior. Plants of the *Rydbergii-strigosa* assemblage are found in the eastern and northern Rocky Mountain area and across the great plains, perhaps also as far west as the state of Washington. These plants, of which the chromosome configurations of 21 races have been examined, have shown without exception a circle of 14. All so far analyzed are true-breeding complex-heterozygotes, with balanced lethals and small or moderately small self-pollinated flowers. In these respects, they resemble races in the eastern part of the range. They have one distinctive cytogenetic feature in common with *irrigua*, however, namely the fact that both complexes in a given race (so far as analyzed) are fairly closely related to the *hookeri* complexes, as shown by the fact that they give chromosome configurations of intermediate nature when combined with the latter. In the *strigosa-Rydbergii* group, however, the two complexes making up a race are less closely related to each other segmentally than they are to the presumed common ^{h}*hookeri*-like ancestor, whereas in *irrigua* they are about as closely related to each other as they are to the ancestral complex. In the one case, the complexes in a race represent the result of divergence in radically different directions; in the other case they have resulted from divergence in more or less similar directions. In spite of the marked differences in chromosome configuration as between the *irriguas* and the *strigosa-Rydbergii* group, however, these two groups are no doubt related in about the same degree to the *hookeris*. In different ways, both groups show phenotypic resemblance to the *hookeris*, also.

So far as our studies show, therefore, it may be said that the *irriguas* and the *strigosa* and *Rydbergii* forms have each their own distinguishing cytogenetic characteristics which may prove of definite diagnostic value. They both show on the basis of segmental resemblance a fairly close affinity to the *hookeris*, in the case of both complexes.

Another group of races that may be mentioned is the *Jamesiis*. These

differ among themselves in many ways but are all large plants with flowers about ten centimeters across, having extremely long hypanthia. Two races from Oklahoma have shown chromosome configurations of an intermediate nature, like the *irriguas*, and one of these, crossed with various *hookeri* complexes, gives hybrids with intermediate configurations. These are probably related fairly closely to the *hookeris*. A third race from Mexico has seven pairs. It is probably, if anything, more closely allied to the *hookeris* than are the others, although this is still to be tested cytologically.

We shall now turn our attention to the more easterly portion of the range. We find that practically all races east of the great plains have a circle of 14. A total of 47 such races has been examined; of these one has shown all-pairing chromosomes; all but four of the remaining 46 races have shown a circle of 14. All forms with a circle of 14 so far tested are permanent heterozygotes, breeding true because of balanced lethals. All are self-pollinating, and flower size ranges from small to intermediate.

These races have been sent me under a variety of names or under no name at all. They represent a great variety of types, classification of which on purely phenotypic grounds is extremely difficult. When cytological work was begun on these forms it at first looked as though the cytological situation would prove as hopelessly tangled and complex as the phenotypic situation. Every complex seemed to be different and distant from every other one. During the past year, however, evidence has begun to come in which gives us reason to hope that the situation will not prove to be beyond analysis. We are beginning to recognize certain great groups of races which are not only phenotypically similar, but which are characterized by the possession of certain distinctive associations of complexes. As yet this evidence is meagre and the outlines of the picture are vague and incomplete, but we now have hopes of ultimately analyzing them, as will be seen from the following.

During the past two years, Dr. B. L. HAMMOND, my assistant, has been specializing upon forms which fall under the name of "*biennis*" in the manuals. He finds that these, so far as examined, have a distinctive situation with regard to their complexes. They have one complex (usually the α or egg complex) which has a segmental arrangement closely allied to the *hookeri* arrangements and another (usually the β or pollen complex) whose segmental arrangement shows much less resemblance to the *hookeri* complexes. The former complex is responsible for most of the "*biennis*" characters, for example, broad, crinkly leaves; the other produces narrow foliage, and in general an effect entirely different from that seen in *biennis*. The specific segmental arrangements may vary from race to race, but in four races, three from various points in Kentucky (a middle western state), the fourth from Maryland, the α complexes are identical in segmental ar-

rangement, and the β complexes are also identical. Further study will show whether this cytogenetic situation is characteristic of all *biennis* forms. It has been found so far in 10 races, and several additional strains which have been only partially studied seem to be showing the same situation. We are inclined to expect, therefore, that the presence of a broad-leaved complex closely related to the *hookeri* complexes in segmental arrangement, and a narrow-leaved complex unrelated to the *hookeris* may prove to be characteristic of all forms of this (the *biennis*) group.

It may be added that the behavior of the so-called "gamete lethals" varies from race to race within the *biennis* group. Some of these races are strictly heterogamous, some are isogamous, and some show intermediate conditions. There are one or two in which the broad-leaved complex is the pollen (β) complex, and the narrow-leaved one is the egg (α) complex. In every case, so far, however, it is the broad-leaved complex which is related segmentally to the *hookeris*.

Another group which shows indications of having a characteristic association of complexes is the *grandiflora* group, of which my strains have all come from the southern coastal plain (Alabama, Mississippi, and Florida). This group has thus far shown a similar situation to the *biennis* group, in that one complex is closely allied segmentally to the *hookeris*, the other is unrelated. The complexes of the *grandifloras*, however, produce phenotypic effects quite different in many particulars from the *biennis* complexes and have perhaps developed quite independently of the latter. I have not gone deeply enough into this group to say more at present.

A third group which seems to belong to *nutans* and *laevigata* has been investigated in a preliminary way only, and the results are not entirely clear. Indications are that both complexes in these forms produce broad, fairly crinkled leaves and slender buds, but that one complex produces these effects more strikingly than the other. Early returns from the cytological investigations suggest that both complexes may bear a fairly close relationship to the *hookeris*. There has not been time as yet to compare them with the *biennis* and *grandiflora* complexes.

This, in barest outline, is the general situation as far as it is known at present. Much work remains to be done on the groups mentioned, and several other groups await investigation. It seems clear, so far, that the *hookeris*, the *irriguas*, the *Rydbergiis* and *strigosas* and possibly the *biennis* and *grandiflora* groups have their own characteristic types of complex from the standpoint of segmental arrangement. It is perhaps not too much to expect that other groups will also show distinctive features.

We may now ask how the situation found throughout most of the range of Onagra is to be interpreted. How has the union of diverse complexes into a plant possessing a chain of 14 chromosomes come about, and what

is the reason for the survival, with few exceptions, of only those plants with diverse complexes and a circle of 14? In this connection, one fact seems to stand out as of particular importance, namely, that the complexes within a race often show less affinity for each other segmentally and genetically than they do for certain complexes in other races; this strongly suggests that hybridization has played an important role in the production of the various races. Through natural crossing, diverse complexes have been brought together. Many of these hybrids have bred true, owing to the lethals present, and have become established as true-breeding races or species. We are confronted, therefore, with the possibility that a large percentage of the Onagra population is actually of hybrid origin.

But we know from hybridization experiments that there is at least an equal chance that crossing will produce hybrids with some other configuration than a circle of 14. Why have only those with a circle of 14, with rare exceptions, survived? The answer is perhaps to be found in the suggestion (cf. DARLINGTON, 1929) that this configuration has survival value because it maintains a maximum of heterozygosity with a minimum of resultant sterility. We may state the case in this way: self-pollination is theoretically the surest and most efficient method of ensuring the setting of seed, even in large-flowered forms, but especially in those which come to have small flowers. It has, however, the disadvantage of reducing heterozygosity and thus depriving the plant of the benefits of hybrid vigor. Furthermore, as long as the chromosomes remain paired, balanced lethals are of little value, in fact are disadvantageous because they cut down fertility. Balanced lethals in a single chromosome pair would preserve the heterozygosity of this one pair alone, and would at the same time cut normal fertility in two. If additional chromosome pairs had balanced lethals, the proportion of genes preserved in the heterozygous condition would be correspondingly increased, but half of the remaining fertility would be lost for every additional chromosome pair having lethals. Thus, if all seven pairs in an all-pairing Onagra had balanced lethals, fertility would theoretically be reduced to less than 1 percent. Should, however, all or most of the chromosomes come to be linked into one chain, a single pair of lethals would preserve the heterozygosity of the whole or nearly the whole set of genes, with only 50 percent sterility. With heterozygosity of all or practically all the chromosomes thus preserved, cross-pollination would no longer be of value, and the increased certainty of seed setting resulting from self-pollination would probably more than make up for the sterility due to the lethals.

Hence it is evident that the presence of a circle of 14 has come to have a unique survival value. It has given to the plants possessing it an advantage in the struggle for existence, for it has permitted the setting of a

plentiful supply of seed and at the same time has ensured to the plant hybrid vigor in a greater degree than is available to plants which have fewer chromosomes covered by balanced lethals. Hybrids with other configurations than a circle of 14 have therefore been largely weeded out. We see also why it is that balanced lethals have been found only in races with large circles.

In this brief statement it has been possible only roughly to indicate the course of our studies and to suggest the nature of the results which are being obtained. Detailed analyses of segmental arrangements in the various complexes and a full discussion of the results of these analyses will be presented in succeeding papers.

SUMMARY

Certain cytogenetically rather well-defined groups of races are now becoming recognized which may be called tentatively by the names *hookeri*, *irrigua*, *strigosa*, *biennis*, etc. In a given race of *hookeri*, associated complexes are identical or similar in segmental arrangement, and this is true also of complexes in different races of *hookeri*. In the *irriguas*, associated genoms are more or less similar, frequently giving chromosome configurations which include one or more small or medium-sized circles. These genoms show the same degree of relationship in segmental arrangement to the *hookeri* genoms that they do to each other. In the *strigosas*, associated complexes are quite unlike in segmental arrangement, giving a circle of 14, but both complexes in a given strain are in this regard rather closely related to the *hookeris*. In each of the *biennis* forms so far studied, the complex producing broad leaves is closely related in segmental arrangement to the *hookeri* complexes; the opposing complex, which produces narrow leaves, is less closely related. Other groups, which are less thoroughly studied, seem also to show characteristic features. Facts such as these, when they become more numerous, should help us to discover such natural groupings as exist in the Onagras and to understand how these various groups are related to one another.

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THE TIME OF EFFECT OF SOME SECOND CHROMOSOME LETHALS IN DROSOPHILA MELANOGASTER

GEORGE BRODY
Harvard University, Cambridge, Massachusetts

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THE purpose of this study was to determine which of a series of lethals are effective in the egg stage.

Six different lethals, carried as Curly balanced stocks, were obtained from Dr. PLOUGH of Amherst College. Each of these lethals, numbered 1, 4, 9, 11, 19, 21, was isolated from wild Belfast-Maine stock in the fall of 1938. The lethals are not associated with inversions or translocations; it has not yet been determined if they are associated with deficiencies. They are all located in the second chromosome.

In a later paper experimental methods will be described in some detail. For the collection of eggs the techniques described by the writer (1939) were used. Where later stages of development (that is, larval and pupal) were not to be studied, it sufficed to align the eggs collected at the end of twenty-four hour intervals in rows of ten on the surface of the medium used for egg collection. When later stages were to be followed, eggs were transferred to strips of black photographic paper lying on the surface of the medium in Petri dishes and again aligned in rows of ten. In both cases, counts were made after twenty-four hours and again after forty-eight hours to determine embryonic mortality. The medium was liberally supplied with yeast so that lack of food would not be a factor in larval mortality. All work was carried out at 25°C.

EXPERIMENTAL DATA

For the data in table 1, the eggs laid by $l/+\times l/+$ (outcrossed with Canton +) were counted first to eliminate mortality possibly due to the "Curly" lethal. Canton+ egg counts were used as controls to determine "normal" embryonic mortality (residual mortality). MSC, an Amherst wild strain, mass cultured for two years, was also tested. No significant difference was found for embryonic mortality in a comparison between counts of sib matings, $l/+\times l/+$, and Cy/l counts from stock matings. Table 1 lists the data on embryonic mortality of the six lethals from the Belfast-Maine stock in addition to that of the lethal associated with the Cy inversion and of the controls.

Disregarding the residual mortality only l_{21} of the six lethals from the Belfast-Maine stock showed any indication of being effective wholly in the egg stage. The data in table 2 were collected to determine whether any of the embryonic mortality could be ascribed to the lethal associated with the Curly balancer. Table 2 shows the mortality for three developmental

stages of the offspring of the Cy/l_{21} stock, Cy/Canton+ × Cy/Canton+, and Canton+ stock (for an estimate of residual mortality).

TABLE 1

Mortality of different lethals and controls in the embryonic stage.

PARENT GENOTYPE	TOTAL NO. OF EGGS	UNHATCHED EGGS	PERCENT EGG MORTALITY
$l_1/+$	180	24	13.3±2.6*
Cy/l_1	143	24	16.8±3.1
$l_4/+$	319	56	17.5±2.1
Cy/l_4	227	45	19.8±2.6
$l_9/+$	273	62	22.5±2.5
Cy/l_9	350	74	21.2±2.2
$l_{11}/+$	378	49	13.0±1.7
Cy/l_{11}	191	24	12.5±2.4
$l_{19}/+$	362	14	3.9±1.0
Cy/l_{19}	306	26	8.5±1.6
$l_{21}/+$	321	76	23.7±2.4
Cy/l_{21}	1882	492	26.1±1.1
Canton+	595	35	5.9±0.9
MSC+	804	69	8.6±1.0
$Cy/+$**	471	37	7.9±1.2

* Standard error of a percentage.
** Outcrossed with Canton+.

TABLE 2

	Cy/l_{21}	$Cy/+ \times Cy/+$	CANTON+ CONTROLS
eggs observed	1036	471	595
unhatched eggs	261	37	35
percent embryonic mortality	25.2±1.3	7.9±1.2	5.9±0.9*
larvae observed	547	121	500
larvae dead	168	30	33
percent larval mortality	30.7±2.8	24.8±4.0	6.6±1.1
corrected mortality	22.9±1.8	22.9±3.7	6.2±1.1
pupae observed	160	98	213
pupae dead	12	7	5
percent pupal mortality	7.5±1.8	7.2±2.5	1.9±0.9
corrected mortality	3.9±1.5	5.0±2.2	1.7±0.9
Total mortality (corr.)	**52.1%**	**35.7%**	**13.7%**

* Standard error of a percentage.

While the data given above indicate the embryonic mortality of the lethal associated with the particular Curly balancer used, they do not

throw any light on the problem of residual mortality. Residual mortality is the product of two factors, genetic and environmental. Since the environmental factor is presumably uniform in all of the above counts, there remains only the genetic variability of the material to be accounted for in the above data. The frequency of lethals, or possibly the presence of modifying factors in the other chromosomes, determines how much of the residual mortality is due to the genetic condition of the material. The extraneous lethals may be eliminated by inbreeding the balanced stock, but this does not remove the possible influence of modifying factors. Accordingly, Cy/l_{21} females were outcrossed with Canton+ males and with

TABLE 3

GENOTYPE OF PARENTS*	1	2	3	4	5
eggs observed	667	302	477	214	428
unhatched eggs	113	55	22	13	6
percent mortality	16.9±1.5	18.2±2.2	4.6±0.9	6.1±1.4	1.4±0.5
larvae observed	332	247	455	201	206
larvae dead	26	20	111	51	3
percent larval mortality	7.6	8.1	24.4	25.4	1.5
corrected mortality	6.5±1.2	6.6±1.4	23.2±2.0	23.4±2.9	1.4±0.7
pupae observed	306	227	444	150	203
pupae dead	18	13	4	1	2
percent pupal mortality	5.9	5.7	0.9	0.7	1.0
corrected mortality	4.5±1.0	4.3±1.2	0.8±0.13	0.5±0.1	0.9±0.3
Total (corrected)	**27.9±2.2**	**29.1±2.6**	**28.6±2.1**	**30.0±3.1**	**3.7±1.3**

* Legend: 1. $l21$/Canton ♀♀×$l21$/MSC ♂♂
2. $l21$/Canton ♂♂×$l21$/MSC ♀♀
3. Cy/Canton ♀♀×Cy/MSC ♂♂
4. Cy/Canton ♂♂×Cy/MSC ♀♀
5. $l21$/Canton × Cy/Canton

MSC males. Wild type flies of the two F_1's were then crossed with each other and counts were made of the developmental stages of the "synthesized" F_2. The purpose of such a procedure was to reduce as much as possible the genetic residual mortality by increasing heterozygosity in the other chromosomes. The results are tabulated in table 3. "Corrected" percentages are expressed on the basis of original number of eggs.

Some of the dead eggs were dechorionated and examined under the microscope. The eggs were classified as early or late depending on whether tracheal rudiments had been developed. These are formed by the eleventh to twelfth hour according to POULSON (1937). Complete data will be given in a later paper on developmental studies. For the present it suffices to say

that the majority of the wild eggs died before the appearance of tracheal rudiments, that is, were "earlies," while the eggs of both *Cy* and l_{21} were preponderantly "lates."

DISCUSSION

The theoretical expectation for mortality, that is, 50 percent for the balanced Cy/l_{21} stock and 25 percent for the lethal itself, is closely approximated in the data above, if all of the developmental stages are taken into consideration. If, however, we apply the specificity idea to the time of action of lethal genes, we should expect all of the mortality to occur in one of the developmental stages, that is, embryonic, larval, or pupal, for each lethal in question. That none of the lethals studied adheres closely to this rule is obvious from the data presented. While the data of table 1 would seem to indicate that as far as $l21$ is concerned, the theoretical expectation is adequately fulfilled ($l21$ expressing itself in the egg stage and *Cy* in the larval stage) the data of table 3 show otherwise. The apparent fulfillment of expectation would disregard residual mortality. Thus, the data of table 1 when corrected for residual mortality are comparable to that of table 3. In the case of $Cy/l21$ the embryonic mortality, when corrected for residual mortality, becomes 18.2 percent compared to 16.9 percent and 18.2 percent from table 3. While the data available for the later stages of $l19$ are insufficient for tabulation, it may be said that of the six lethals studied, it alone comes close to being stage specific, most of the mortality occurring in the larval stage.

This apparent lack of specificity has been evident in earlier reports on the time of effect of lethals (DEMEREC and HOOVER (1936), BREHME (1937), KALISS (1939), and others). On the other hand, many other lethals seem to be specific as reviewed by STERN (1939). The data given above for the time of effect of the lethal associated with the particular Curly inversion used as balancer in this study agree with those of SIVERTZEV-DOBZHANSKY (1927). Although she interpreted the data as indicating that Curly itself was the lethal, she found that death occurred chiefly in the second instar. Presumably the two stocks carry the same lethal. It will be noted that the figures obtained by the writer for Canton+mortality agree very well with those obtained by BREHME (1937) for Florida +. The problem of residual mortality has been attacked by many investigators, STURTEVANT and BEADLE (1936), SCHWEITZER and KALISS (1935), and others. In general it may be said that residual mortality varies from about three percent to about eight percent, depending probably more on the genetic composition of the stock than on environmental factors. The lower limit of residual mortality is obviously that set by mutation frequency, and it is therefore impossible to eliminate it entirely. The extreme environmental factors

which may increase residual mortality, and which are rarely encountered in a well conducted experiment, are dessication in the case of eggs not laid on the medium, and suffocation by overgrowth of yeast.

Several explanations, or rather speculations, may be offered for the apparent lack of specificity of time of action manifested by the above and other lethals. We may assume the presence of other lethals or modifying factors either in the chromosome studied or in the other chromosomes. While the technique of outcrossing to increase heterozygosity does not eliminate entirely this possibility, it reduces it considerably. The factors which would be most difficult to eliminate are those present on the same chromosome and closely linked to the lethal being studied. However, if we assume random distribution for such factors throughout the whole chromosome complement, their influence becomes negligible compared to the obtained results. There remains then one other explanation.

Heretofore, we have been separating arbitrarily the egg stage or so-called embryonic stage from the larval stage, while actually the newly eclosed larva is already in the first instar, that is to say, development up to the first ecdysis is one continuous process. We may suppose that development, at least as far as most larval organs (with the possible exception of those lost at each moult) and imaginal anlage or discs are concerned, is a continuous process through all of the "stages of development." This statement should perhaps be limited for the larval organs, where development is continuous at least until the pupal period, when they degenerate. This may perhaps explain the infrequency of pupal lethals. Now if we postulate that some lethals have a wider range of Gaussian variation than others, we may think of the "specific" time of action as being the modal time of action. Where there is a deficiency of some substance necessary for a transition from one stage to another, we may expect to find this curve of variation present up to the transition point and then abruptly broken off. Thus, for example, if the lytic enzyme necessary for the rupture of the vitelline membrane prior to eclosion is absent, or if a hormone (or its precursor, which is ultimately of genic origin) necessary for an ecdysis or pupation is absent, development in terms of "stages" terminates abruptly and we have an apparently specific time of action. The variation which precedes this event is normal in every respect. The eggs studied after dechorionation manifested this normality of variation to such an extent, that for the purposes of this study it was necessary to set up an arbitrary line of classification in the time scale (that is, eleventh to twelfth hour—see above, experimental data). Of those eggs which died later than the arbitrary time, none had mouth parts which had turned black, although many individuals had developed fully to pre-eclosion larvae and still showed signs of motion in wet mount preparations. It is suspected that one of the factors involved in this

lethality is an upset of the tyrosinase-oxidase system. Respiration as well as developmental studies have been begun.

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SUMMARY

1. Seven lethals were studied to observe their embryonic mortality. They showed different amounts of embryonic mortality varying from 2 percent below the wild type control to 19.3 percent above it.

2. The evidence seems to indicate that a lethal may manifest itself at any stage of development with no specific time of action at which all of the homozygotes of a particular lethal die. Attempts to eliminate extraneous factors which possibly bring about this condition of "non-specificity" show even more conclusively the existence of such "non-specificity."

3. Possible explanations for this apparent lack of specificity are given.

4. The lethal associated with the particular Curly inversion used as balancer in this study manifests itself chiefly in the second instar, and is apparently the same as that used by SIVERTZEV-DOBZHANSKY (1927) in her studies.

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